



Original research

Stromal cell derived factor-1, CXCR4 and CXCR7 gene transcripts in pterygia

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Received 2 August 2016; revised 26 October 2016; accepted 30 October 2016

Available online 27 December 2016

Abstract

Purpose: Pterygium is a pathologic process with angiogenic and tumor cell like characteristics. Chemokine and chemokine receptors may contribute to the formation and growth of pterygia. The aim of this study was to assess the expression of stromal cell derived factor (SDF)-1, as an angiogenic chemokine, and its receptors, CXCR4 and CXCR7, gene transcripts in pterygia.

Methods: RNA was extracted from tissue samples of 33 patients with primary pterygium and 35 volunteers with conjunctiva as the control group. Then the mRNA expression of SDF-1, CXCR4, and CXCR7 was assessed through quantitative Real Time PCR method using appropriate primers.

Results: SDF-1 and both receptors transcripts had significantly higher expression in pterygia samples compared to the control group ($P < 0.05$). The ratio of CXCR7 transcript expression to CXCR4 was 26.4 in patients while it was 11 in controls.

Conclusion: As SDF-1 and its receptors, CXCR4 and CXCR7, were up-regulated in pterygia, SDF-1/CXCR4/CXCR7 axis may contribute to pterygium formation which can be possibly restrained by down-regulating this signaling pathway.

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Keywords: Pterygium; SDF-1; CXCR4; CXCR7; Angiogenesis

Introduction

Pterygium is one of the most common ocular disorders with irritating symptoms and important effects on the corneal regularity and visual acuity. Discrepancy in prevalence was seen geographically by higher rates of occurrence in dry and dusty climate especially in areas named as pterygium belt.^{1,2} Pterygia and ocular surface squamous neoplasia (OSSN) have shared risk factors and may simultaneously occur in a person. The highest rate of OSSN, 9.8% in patients with pterygium after excision, was reported in Australia.^{3,4} In contrast, Yeung et al found no case of OSSN in 1127 pterygium specimens.¹

Pterygium is known as a pathologic process accompanied with proliferation of epithelial cells, collagen remodeling,

angiogenesis, and inflammation.⁵ Moreover, angiogenesis plays important roles in pathogenesis of pterygium, and several reports advocate the role of angiogenic mediators in the progression and growth of a pterygium.^{6–9}

Chemokines and their receptors are a group of small proteins which are responsible for diverse physiological and pathological responses. CXCL12 (SDF-1, stromal cell derived factor-1) is one of the CXC chemokines that involves biological events such as organogenesis through binding to its receptors, CXCR4 and CXCR7.^{10,11}

The SDF-1-CXCR4 axis primarily involves neural, vascular, and craniofacial organogenesis.¹² This pathway has the ability to control cell cycle and to regulate apoptosis by producing effector molecules such as NF- κ B.^{11,13} Another important effect of this pathway is its role in angiogenesis. Endothelial progenitors and mature endothelial cells express CXCR4 which is up-regulated by inflammatory and angiogenic factors.^{14,15}

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Peer review under responsibility of the Iranian Society of Ophthalmology.

New advances in cancer metastasis research revealed SDF-1/CXCR4/CXCR7 roles in the recruitment of endothelial progenitor cells, angiogenesis, and spread of tumor cells.^{16–19} CXCR4 expression results in progression, increased size of tumor and metastasis in breast cancer and oral squamous cell carcinoma (SCC).^{20,21} In addition, reports demonstrated SDF-1/CXCR4 axis as a susceptibility factor for lung, breast, and prostate cancers.^{22–25}

According to the behavior of pterygium as a proliferative process with tumor-like characteristics and the role of SDF-1/CXCR4/CXCR7 axis in tumor initiation and angiogenesis, we aimed to investigate the expression of this chemokine-receptors axis in pterygium compared to a control group.

Methods

In this prospective, comparative study, pterygium samples of 33 (12 males and 21 females) patients were referred to Stem Cell Laboratory of Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences from Khalili Hospital during the years 2012–2013. Conjunctival samples from 35 sex-matched volunteers (13 males and 22 females) undergoing cataract surgery without any sign of pterygium or pinguecula, ocular surface pathology, and no history of previous ocular surgery were used as the control group. Samples from the case group were obtained from nasal pterygium, and all samples of the control group were obtained from nasal conjunctiva 2 mm from limbus. The mean age of patients and controls were 51.9 ± 15 and 64.2 ± 14 years, respectively ($P = 0.004$). All participants provided their informed consent to take part in this study, and the Ethics Committee of Shiraz University of Medical Sciences approved the study (Ethical code: EC-P-92-5375). All samples were stored at -70°C refrigerator before experiments.

RNA isolation and cDNA synthesis

Total RNA was isolated from 2 mm tissue specimens using RNA isolation kit (Roche, USA) based on the manufacturer's instructions. The quality of extracted RNA was assessed through gel electrophoresis and spectrophotometric analysis, where an A280/A260 of 1.8–2.0 was accepted as a high quality RNA. Then cDNA was synthesized from 5 μg of extracted RNA, using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania).

Quantitative real time PCR (qRT-PCR)

Expression of SDF-1, CXCR4 and CXCR7 gene transcripts were determined by quantitative real-time PCR (qRT-PCR), using an ABI system (Real-time PCR Detector, ABI, USA) with SYBR Green I PCR Master Mix kit (Applied Biosystems, USA). Each PCR reaction was performed in a final volume of 25 μl containing 0.5 μg of the cDNA products, $1\times$ reaction mixture and 10 pmol of reverse and forward primers designing by Primer 3 open source software (Sourceforge, USA). Thermal cycling for all genes was initiated with an initial

denaturation step at 95°C for 10 min, followed by 50 cycles with denaturation at 95°C for 20 s, annealing at 58°C for 20 s, and extension at 60°C for 34 s. 18S rRNA housekeeping gene was used as a reference for the level of target gene expression. The qRT-PCR amplification products were analyzed by melting curve analysis.

Statistical analysis

The relative amounts of SDF-1, CXCR4 and CXCR7 transcripts were determined using $2^{-\Delta\Delta\text{CT}}$ method. Expressions of these genes in patients were compared to the corresponding values from control group using non parametric Mann-Whitney U test by SPSS software v. 15.0 (SPSS, Chicago, IL, USA), and $P < 0.05$ was considered significant. The correlation between gene transcripts and the sex and ages of patients and controls were determined by Spearman's rank parallel test. Relative expression was plotted using GraphPad Prism 5 software (Inc; San Diego CA, USA, 2003).

Results

SDF-1 gene expression

SDF-1 transcripts showed 9.4-fold statistically significantly higher expression in pterygium tissues compared to control tissue samples ($P = 0.02$, Fig. 1, Table 1).

The assessment of relationship between the gene transcripts and ages as well as the gender of patients or controls showed no significant correlation (all $P > 0.05$).

CXCR4 gene expression

As depicted in Fig. 2 and Table 1, patients with pterygium expressed significantly increased level of CXCR4 mRNA (116-fold) compared to control group ($P = 0.0001$). Median of

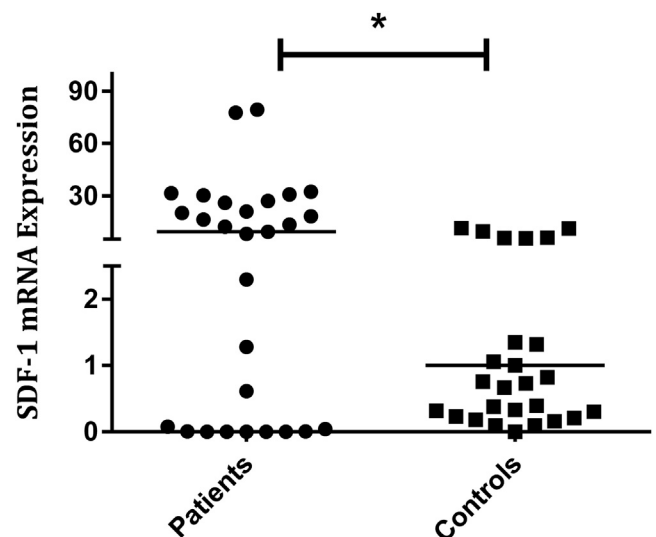


Fig. 1. Expression of stromal cell derived factor-1 (SDF-1) mRNA in patients with pterygium and control individuals. Data are shown as the median of $2^{-\Delta\Delta\text{CT}}$. * shows $P < 0.05$.

Table 1
The amount of stromal cell derived factor-1 (SDF-1), CXCR4 and CXCR7 gene transcripts in patients and controls.

	Patients	Controls	P value
Sex	12 males & 21 females	13 males & 22 females	1
Age	51.9 ± 15	64.2 ± 14	0.004
SDF-1	Median of 2 ^{-ΔCT} 10 ³ Mean of 2 ^{-ΔCT} 10 ³ ± SEM	0.006 0.015 ± 0.003	0.02
CXCR4	Median of 2 ^{-ΔCT} 10 ³ Mean of 2 ^{-ΔCT} 10 ³ ± SEM	0.00203 0.019 ± 0.01	0.0001
CXCR7	Median of 2 ^{-ΔCT} 10 ³ Mean of 2 ^{-ΔCT} 10 ³ ± SEM	0.06 0.14 ± 0.05	0.0001

SDF-1: stromal cell derived factor-1.

2^{-ΔCT}10³ for CXCR4 were 0.236 and 0.00203 in patients and controls, respectively. However, no significant correlation was found between CXCR4 mRNA expression and the sex and ages of patients and controls (all *P* > 0.05).

CXCR7 gene expression

Evaluation of CXCR7 gene transcripts showed significant higher expression of this chemokine receptor in cases compared to controls (*P* = 0.0001). CXCR7 had 282-fold higher expression of mRNA in patients than individuals with conjunctiva (Fig. 3 and Table 1). No significant correlation was found between CXCR7 mRNA expression and the sex and ages of patients and controls (all *P* > 0.05).

Assessment the correlation between CXCR4 and CXCR7 transcript expression showed a positive but non-significant correlation (spearman *r* = 0.2, *P* > 0.05).

CXCR7 to CXCR4 expression ratio in patients and control individuals

Expression of CXCR7 transcripts was 26.4-fold higher than CXCR4 mRNA in patients with pterygia whereas it was 11 in conjunctival samples.

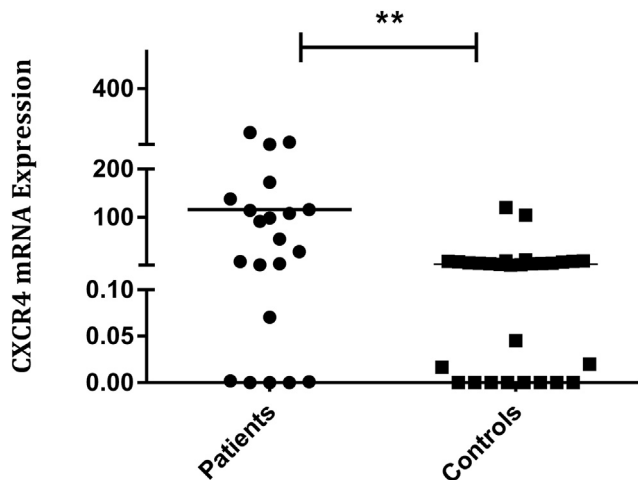


Fig. 2. CXCR4 transcript expression in patients with pterygium and control individuals. Data are shown as the median of 2^{-ΔCT}. ** shows *P* < 0.01.

Discussion

This study revealed high expression of SDF-1, CXCR4, and CXCR7 in pterygium tissues compared to normal conjunctiva. This increase may result in premalignant behavior and recurrence of pterygium although more evidence is needed to confirm this hypothesis.

Pterygium as a general pathologic process of ocular surface expresses tumor-like characteristics. Reisman et al showed low expression of P53 in pterygium as a result of UV radiation. Lack of this tumor suppressor gene led to defective apoptosis in response to UV induced DNA damage.²⁶ In addition, Davanger and Evensen described pterygium as an alteration in limbal stem cells²⁷ and since then, many investigations were performed on this attractive concept. Recently, Chui and colleagues described pterygium as a disease of limbal stem cells. They concluded that the clinico-pathologic process of pterygium happens through this cluster of altered stem cells.⁹

Increasing number of investigations emphasized the role of SDF-1/CXCR4/CXCR7 axis in tumor metastasis and prognosis. SDF-1 polymorphism was reported as a risk factor for

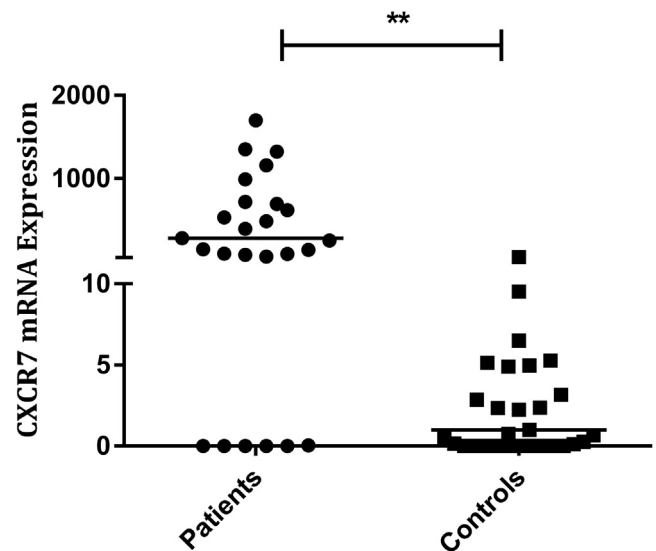


Fig. 3. Expression of CXCR7 gene transcripts in patients with pterygium and control individuals. Data are shown as the median of 2^{-ΔCT}. ** shows *P* < 0.01.

developing breast, lung, colorectal, and prostate cancers.^{22–24,28} The polymorphisms of SDF-1 and CXCR4 are determined as susceptibility markers and prognostic factors for non small cell lung cancer.²⁹ Mirisola et al reported SDF-1 expression as an independent prognostic marker of breast cancer.³⁰ Cronin et al reported the hypoxia dependent pathologic process through which expression of CXCR4 leads to breast cancer metastasis.⁷ SDF-1 axis has also critical roles in angiogenesis as Silva and colleagues showed the effect of this axis on new vessel formation in ischemic retina and choroidal neovascularization in age-related macular degeneration. They concluded that even in the absence of hypoxia, the presence of VEGF recruits this pathway for neovascularization.⁸ Another study showed that neovascularization can be induced by subcutaneous injection of SDF-1 in mice.¹⁵ Down-regulation of angiogenesis has been found to be accompanied with lower level of SDF-1 in idiopathic pulmonary fibrosis.^{31,32}

According to these findings showing the function of SDF-1 axis in angiogenesis and the fact that angiogenesis plays important roles in the growth of a pterygium,³ overexpression of SDF-1, CXCR4 and CXCR7 in our pterygium samples compared to control group probably has crucial roles in the pathogenesis of pterygium. It should be noted that interaction of SDF-1 with CXCR4 or CXCR7 leads to different downstream events.^{33–35} SDF-1/CXCR4 axis activation results in chemotaxis and recruitment of tumor stem cell like cells,^{36,37} whereas SDF-1/CXCR7 signaling pathway can stimulate proliferation³⁸ and vascularization.³⁵ In addition, SDF-1 through both receptors, CXCR4 and CXCR7, contributes to the production of other angiogenic factors such as IL-8 and MMP-3 by tumor cells in different types of tumors such as glioma, breast carcinoma, and lung cancer.^{39,17} Based on the results of our study, SDF-1 may mediate the growth and recurrence of pterygium through both receptors. Consistently, Kim and coworkers reported higher expressions of SDF-1 and CXCR4 in grade T3 pterygium tissues and perivascular regions of pterygium, respectively. They found a significant correlation between the severity of pterygia and SDF-1/CXCR4 signaling pathway.⁴⁰ As we found higher expression of CXCR7 to CXCR4 in patients compared to control individuals, we conclude that CXCR7 may have more important roles than CXCR4 for the growth and recurrence of pterygia.

We note some limitations to our study. First, the sample size was small, and we did not categorize the pterygia based on morphologic and pathologic characteristics. Second, we did not follow-up the patients to record the recurrence of pterygia and its correlation with the expression level of SDF-1, CXCR4 and CXCR7 gene transcripts. Finally, the studied groups were not age-matched, and this may be a limitation for interpretation of the results.

In conclusion, the results of our study may identify new outlooks for the role of SDF-1 and its receptors in pterygium pathogenesis which motivates us to follow pterygium after surgical removal. Moreover, SDF-1/CXCR4/CXCR7 axis may

be aimed as an effective target for adjunctive therapy especially in recurrent pterygium.

Acknowledgement

This work was supported by grants from Shiraz University of Medical Sciences [Grant No. 91-01-01-5375] and Shiraz Institute for Cancer Research [ICR-100-504]. This research was done as a requirement for the Medical thesis defended by Dr. Atta Taseh.

References

1. Yeung SN, Kim P, Lichtinger A, et al. Incidence of ocular surface squamous neoplasia in pterygium specimens: an 8-year survey. *Br J Ophthalmol*. 2011;95:592.
2. Duke-Elder S, ed. *Diseases of the Outer Eye Part 1*. System of Ophthalmology 8. London: Kimpton; 1965:569–585.
3. Hirst LW, Axelsen RA, Schwab I. Pterygium and associated ocular surface squamous neoplasia. *Arch Ophthalmol*. 2009;127:31–32.
4. Oellers P, Karp CL, Sheth A, et al. Prevalence, treatment and outcomes of coexistent ocular surface squamous neoplasia and pterygium. *Ophthalmol*. 2013;120:445–450.
5. Wang IJ, Lai WT, Liou SW, et al. Impression cytology of pterygium. *J Ocul Pharmacol Ther*. 2000;16:519–528.
6. Bradley JC, Yang W, Bradley RH, Reid TW, Schwab IR. The science of pterygia. *Br J Ophthalmol*. 2010;94:815–820.
7. Cronin PA, Wang JH, Redmond HP. Hypoxia increases the metastatic ability of breast cancer cells via upregulation of CXCR4. *BMC Cancer*. 2010;10:225.
8. Lima e Silva R, Shen J, Hackett SF, et al. The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization. *FASEB J*. 2007;21:3219–3230.
9. Chui J, Coroneo MT, Tat LT, Crouch R, Wakefield D, Di Girolamo N. Ophthalmic pterygium a stem cell disorder with premalignant features. *Am J Pathol*. 2011;178:817–827.
10. Mackay CR. Chemokines: immunology's high impact factors. *Nat Immunol*. 2001;2:95–101.
11. Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature*. 2001;410:37–40.
12. Imai K, Kobayashi M, Wang J, et al. Selective secretion of chemoattractants for haemopoietic progenitor cells by bone marrow endothelial cells: a possible role in homing of haemopoietic progenitor cells to bone marrow. *Br J Haematol*. 1999;106:905–911.
13. Helbig G, Christopherson 2nd KW, Bhat-Nakshatri P, et al. NFkappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J Biol Chem*. 2003;278:21631–21638.
14. Murdoch C, Monk PN, Finn A. CXC chemokine receptor expression on human endothelial cells. *Cytokine*. 1999;11:704–712.
15. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells. In vivo neovascularization induced by stromal-derived factor-1alpha. *Am J Pathol*. 1999;154:1125–1135.
16. Vandercappellen J, Van Damme J, Struyf S. The role of CXC chemokines and their receptors in cancer. *Cancer Lett*. 2008;267:226–244.
17. Miao Z, Luker KE, Summers BC, et al. CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci U. S. A*. 2007;104:15735–15740.
18. Kang H, Watkins G, Parr C, Douglas-Jones A, Mansel RE, Jiang WG. Stromal cell derived factor-1: its influence on invasiveness and migration of breast cancer cells in vitro, and its association with prognosis and survival in human breast cancer. *Breast Cancer Res*. 2005;7:R402–R410.

19. Wagner PL, Hyjek E, Vazquez MF, et al. CXCL12 and CXCR4 in adenocarcinoma of the lung: association with metastasis and survival. *J Thorac Cardiovasc Surg.* 2009;137:615–621.
20. Luker KE, Luker GD. Functions of CXCL12 and CXCR4 in breast cancer. *Cancer Lett.* 2006;238:30–41.
21. Uchida D, Begum NM, Tomizuka Y, et al. Acquisition of lymph node, but not distant metastatic potentials, by the overexpression of CXCR4 in human oral squamous cell carcinoma. *Lab Invest.* 2004;84:1538–1546.
22. Razmkhah M, Doroudchi M, Ghayumi SM, Erfani N, Ghaderi A. Stromal cell derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. *Lung Cancer.* 2005;49:311–315.
23. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res.* 2002;62:1832–1837.
24. Razmkhah M, Talei AR, Doroudchi M, Khalili-Azad T, Ghaderi A. Stromal cell-derived factor-1 (SDF-1) alleles and susceptibility to breast carcinoma. *Cancer Lett.* 2005;225:261–266.
25. Shen W, Cao X, Xi L, Deng L. CXCL12 G801A polymorphism and breast cancer risk: a meta-analysis. *Mol Biol Rep.* 2011;39:2039–2044.
26. Reisman D, McFadden JW, Lu G. Loss of heterozygosity and p53 expression in Pterygium. *Cancer Lett.* 2004;206:77–83.
27. Davanger M, Evensen A. Role of the pericorneal papillary structure in renewal of corneal epithelium. *Nature.* 1971;229:560–561.
28. Hidalgo-Pascual M, Galan JJ, Chaves-Conde M, et al. Analysis of CXCL12 30UTR G/A polymorphism in colorectal cancer. *Oncol Rep.* 2007;18:1583–1587.
29. Lee YL, Kuo WH, Lin CW, et al. Association of genetic polymorphisms of CXCL12/SDF1 gene and its receptor, CXCR4, to the susceptibility and prognosis of non-small cell lung cancer. *Lung Cancer.* 2011;73:147–152.
30. Mirisola V, Zuccarino A, Bachmeier BE, et al. CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of disease-free and overall survival. *Eur J Cancer.* 2009;45:2579–2587.
31. Antoniou KM, Soufla G, Lymbouridou R, et al. Expression analysis of angiogenic growth factors and biological axis CXCL12/CXCR4 axis in idiopathic pulmonary fibrosis. *Connect Tissue Res.* 2010;51:71–80.
32. Grunewald M, Avraham I, Dor Y, et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell.* 2006;124:175–189.
33. Fulton AM. The chemokine receptors CXCR4 and CXCR3 in cancer. *Curr Oncol Rep.* 2009;11:125–131.
34. Liberman J, Sartelet H, Flahaut M, et al. Involvement of the CXCR7/CXCR4/CXCL12 axis in the malignant progression of human neuroblastoma. *PLoS One.* 2012;7:43665.
35. Würth R, Barbieri F, Bajetto A, et al. Expression of CXCR7 chemokine receptor in human meningioma cells and in intratumoral microvasculature. *J Neuroimmunol.* 2011;234:115–123.
36. Bleul CC, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature.* 1996;382:829–833.
37. Hattermann K, Held-Feindt J, Lucius R, et al. The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates anti-apoptotic effects. *Cancer Res.* 2010;70:3299–3308.
38. Burns JM, Summers BC, Wang Y, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med.* 2006;203:2201–2213.
39. Ping YF, Yao XH, Chen JH, et al. The anti-cancer compound Nurdy inhibits CXCR4-mediated production of IL-8 and VEGF by malignant human glioma cells. *J Neurooncol.* 2007;84:21–29.
40. Kim KW, Park SH, Lee SH, Kim JC. Upregulated stromal cell-derived factor 1 (SDF-1) expression and its interaction with CXCR4 contribute to the pathogenesis of severe pterygia. *Invest Ophthalmol Vis Sci.* 2013;54:7198–7206.