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MULTI-AUTHOR REVIEW

Periostin in vitreoretinal diseases

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Abstract Proliferative vitreoretinal diseases such as diabetic retinopathy, proliferative vitreoretinopathy (PVR), and age-related macular degeneration are a leading cause of decreased vision and blindness in developed countries. In these diseases, retinal fibro(vascular) membrane (FVM) formation above and beneath the retina plays an important role. Gene expression profiling of human FVMs revealed significant upregulation of periostin. Subsequent analyses demonstrated increased periostin expression in the vitreous of patients with both proliferative diabetic retinopathy and PVR. Immunohistochemical analysis showed co-localization of periostin with α-SMA and M2 macrophage markers in FVMs. In vitro, periostin blockade inhibited migration and adhesion induced by PVR vitreous and transforming growth factor-β2 (TGF-β2). In vivo, a novel single-stranded RNAi agent targeting periostin showed the inhibitory effect on experimental retinal and choroidal FVM formation without affecting the viability of retinal cells. These results indicated that periostin is a pivotal molecule for FVM formation and a promising therapeutic target for these proliferative vitreoretinal diseases.

Keywords Vitreoretinal disease · Genome-wide gene expression profiling · Proliferative diabetic retinopathy · Proliferative vitreoretinopathy · Age-related macular degeneration · Fibrovascular membranes · Epiretinal membranes · Neovascularization · Fibrosis · Retina · Choroid · Mouse model of oxygen-induced retinal neovascularization · Mouse model of laser-induced choroidal neovascuarization · Single-stranded RNA interference

Gene expression profiling of epiretinal membranes

Proliferative vitreoretinal diseases such as diabetic retinopathy (DR), proliferative vitreoretinopathy (PVR), and age-related macular degeneration (AMD) are a leading cause of decreased vision and blindness in developed countries [1] (Fig. 1). In those diseases, retinal fibro(vascular) membrane (FVM) formation above and beneath the retina plays a pivotal role in the primary pathology [2–4]. The FVM formation reflects a wound healing response, but can be refractory if occurring excessively in the eye [5]. Recent technological advancements in genomics have given investigators new opportunities to identify global gene expression in particular tissues in the eye [6]. Therefore, we sought to develop a novel molecular targeting agent based on the gene expression profiling of human epiretinal FVMs (ERMs).

To identify genes responsible for intraocular proliferation, we first determined the gene expression profiling of human retina, ERMs associated with proliferative diabetic retinopathy (PDR-ERMs), PVR (PVR-ERMs), or less-aggressive secondary ERMs [6–8]. We next determined highly expressed genes in PDR- and PVR-ERMs by comparing the gene expression profiles between PDR-, PVR-ERMs and the retina [7], and genes that determine aggressiveness of

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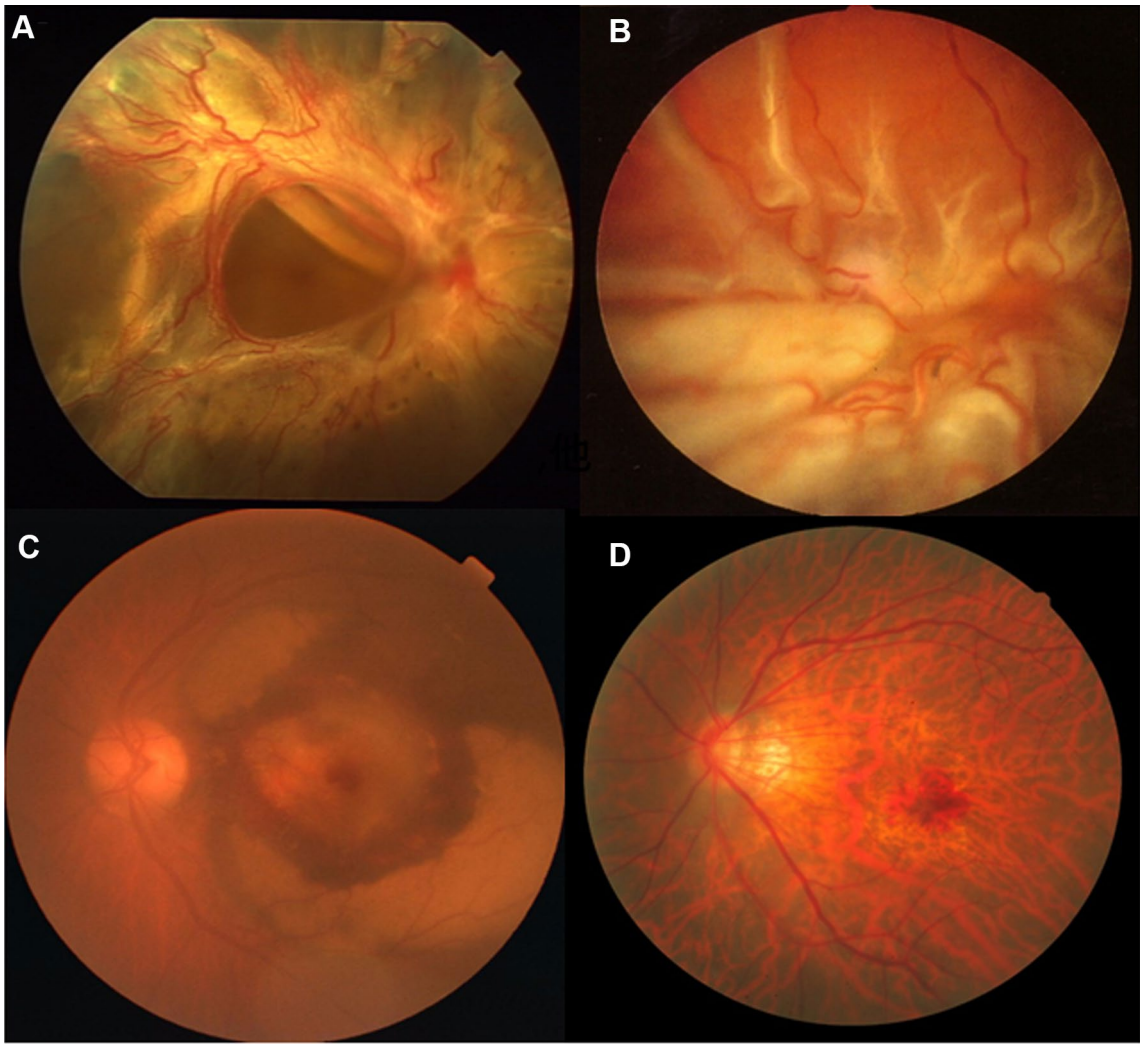

Fig. 1 Proliferative vitreoretinal diseases. **a** Proliferative diabetic retinopathy (PDR). **b** Proliferative vitreoretinopathy (PVR). **c** Age-related macular degeneration (AMD). **d** High myopia

ERMs by comparing the gene expression profiles between PVR-ERMs and less-aggressive secondary ERMs [8]. The former was subdivided by functional subsets of genes related to extracellular matrix, cell adhesion, proliferation, differentiation and other functions, and the latter related to cell proliferation and adhesion. Subsequent analyses identified periostin as a pivotal molecule whose expression is upregulated specifically in proliferating ERMs compared to the retina, and facilitates their proliferation, because it was identified at both comparison procedures.

Periostin, a matricellular protein belonging to the fasciclin family, plays a role in cell motility by interacting with integrin αvβ1, αvβ3, and αvβ5 during tissue development and remodeling. Recent studies have demonstrated that periostin is involved in the development of heart valves, tooth, and bone [9, 10] and tumor metastasis [11]. In tissue remodeling, periostin stimulates regeneration of heart tissue after myocardial infraction [12, 13], cutaneous wound healing [14] and chronic allergic inflammation [15].

Periostin in diabetic retinopathy

Diabetic retinopathy (DR) is one of the leading causes of vision loss in the working-age population worldwide [16]. Retinal neovascularization (NV) arises at the advanced stage of DR leading to proliferative DR (PDR) [17]. Vision loss can result from abnormal FVM formation with subsequent intravitreal hemorrhage and tractional retinal detachment [2]. Despite recent advances in vitrectomy techniques [18–21], usage of retinal laser photocoagulation and intravitreal injection of anti-vascular endothelial growth factor (VEGF), the prognosis for patients with DR is sometimes poor, especially in those with PDR [22].

The mRNA of periostin was detected in all ten of the FVMs obtained from the patients with PDR. In contrast, it was barely detectable in the normal retinas. In addition, RT-PCR yielded multiple bands, indicating the existence of splice variants [23] of periostin in the FVMs. Immunohistochemical analysis exhibited co-localization of α-smooth muscle actin (α-SMA) and periostin in the cells of FVMs [24].

We next examined the amount of periostin in the vitreous samples of PDR patients collected at vitrectomy, and in the vitreous samples obtained from patients during secondary ERM or macular hole (MH) surgery (control) [24]. The concentration of periostin in the vitreous was significantly elevated in the patients with PDR than in the eyes with secondary ERM or MH. The concentration of periostin was significantly associated with the presence of FVMs, suggesting that periostin is closely related to FVM formation in PDR [24].

When we examined the relationship between periostin and VEGF, there was no significant correlation between the vitreous concentrations of periostin and VEGF in the vitreous with PDR [24]. This indicates that periostin and VEGF do not behave in a directly synchronized manner during the development of FVMs. Moreover, compared to VEGF, periostin is presumed to be nonfunctional in normal retinas, because of the very low levels of periostin in the normal control retinas [24].

We also examined the mRNA/protein concentration of periostin in a mouse model of oxygen-induced retinal NV (OIR). The results showed that the level of periostin mRNA/protein in the OIR retinas was significantly elevated at P17 than that in the control retinas [25]. Immunohistochemical analyses of retinal sections exhibited that periostin-positive cells were co-localized with both α-SMA andCD31 in the preretinal pathological NVs. In the retinal flat-mounts, periostin was co-localized with F4/80. Moreover, periostin was co-localized in the preretinal pathological NVs with CD206 [25]. These findings indicated that the expression of periostin was enhanced in the vascular endothelial cells, pericytes, and M2 macrophages in the preretinal pathological NV of OIR retinas.

To investigate whether periostin alters the ischemia-induced retinal NV, we calculated the size of the neovascular tufts and avascular areas in the OIR retinas of wild-type (WT) mice and periostin knockout (KO) mice at P17. In the OIR retinas, the neovascular tufts represent preretinal pathological NV, whereas the avascular areas indicate the physiological revascularization [26]. The size of the neovascular tufts was significantly reduced in the OIR retinas of periostin KO mice than that in WT mice [25]. The mean avascular area was significantly greater in periostin KO mice than that in WT mice [25]. These results suggested that periostin promotes both preretinal pathological NV and physiological NV in OIR retinas.

In vitro experiments using human retinal microvascular endothelial cells (HREC) showed that periostin stimulated the ischemia-induced retinal NV by Akt phosphorylation via integrin αvβ3 [25].

M2 macrophage as a cellular source of periostin

FVMs usually contain different types of cells, such as macrophages/monocytes, hyalocytes, retinal glial cells, fibroblasts, laminocytes and vascular endothelial cells [27]. Among these cells, the macrophages/monocytes have a wide range of biological functions [28, 29]. We have demonstrated that macrophage-attracting chemokines, CCL2, CCL3 and CCL4, played important roles in retinal NV through the recruitment of macrophages/monocytes in a mouse model of OIR [26, 29, 30].

Evidence has been accumulating that macrophages consist of at least two subtypes, classically activated M1 and alternatively activated M2 [31, 32]. The M1 macrophages are proinflammatory and play a pivotal role in driving inflammation, and the M2 macrophages are involved in debris scavenging, NV and fibrosis.

We have demonstrated that there was an increase in the expression of CD163 in the vitreous and FVMs from PDR patients [33]. CD163 is a M2 macrophage marker and showed a close relationship with periostin [33]. The increased expression of CD163 indicated that the M2 macrophages may play a role in the formation of FVMs.

Granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) promote monocyte–macrophage lineage differentiation both in vivo and in vitro [31, 32]. The GM-CSF, or M-CSF-differentiated macrophages can be further polarized to more specific cell types in response to additional stimuli. For instance, when GM-CSF-differentiated M1-like macrophages are exposed to T helper (Th1) cytokines, such as interferon-γ (IFNγ), they are polarized into more activated M1 macrophages and express the M1 cell-surface marker CD80 [34]. In contrast, when M-CSF-differentiated human M2-like macrophages are stimulated by Th2 cytokines, such as interleukin (IL)-4 and/or IL-13, they are polarized into more activated M2 macrophages and express the M2 cell-surface marker CD163 [31].

We have demonstrated that the concentration of M-CSF, but not GM-CSF, was significantly higher in the vitreous of PDR patients than in control patients [35]. An early upregulation of M-CSF signaling of microglia, glia and neurons in the retinas of diabetic rodents has been reported [36], suggesting that a higher concentration of M-CSF in the vitreous of PDR patients is derived from those cells in the diabetic

human retina. In addition, the concentration of M-CSF and soluble(s) CD163 in the vitreous of patients with PDR was significantly correlated [35]. Recently, we demonstrated that CD163-positive M2 macrophages were clustered nearby neovascular tufts in a mouse model of OIR [37]. Along with the predominance of M-CSF over GM-CSF in the PDR vitreous, these findings suggest that diabetic retinas are a M2 macrophage-dominant microenvironment.

The concentration of IL-13 was significantly higher in the vitreous of PDR patients than in control patients, but IL-4 was hardly detectable [35]. IL-13 shares many functional roles with IL-4, because both cytokines exploit the same IL-4Rα/Stat6 signaling pathways [38]. However, recent studies have shown a dominant role for IL-13 in the pathogenesis of several fibrotic diseases such as asthma, pulmonary fibrosis and systemic sclerosis [39]. Consistent with these findings, the concentration of IL-13 was significantly related to the existence of FVMs [35], indicating that IL-13 is closely associated with the formation of FVM in PDR patients. Moreover, the expression of periostin in HRECs was significantly upregulated in a dose-dependent manner only by IL-13 [25]. IL-13 was also expressed by CD4-positive cells in the retinas of OIR.

In addition, a higher correlation between the vitreous levels of M-CSF, sCD163 and periostin in eyes with PDR was detected. Finally, the treatment of M-CSF-differentiated human macrophages by IL-13 resulted in a striking induction of CD163 and periostin with very little upregulations of CD80 [35]. These results suggest that the recruited monocytes in diabetic retina may differentiate into M2-like macrophages by M-CSF, and further polarize to activated M2 macrophages which promote the formation of FVMs by producing periostin (Fig. 2).

Periostin in proliferative vitreoretinopathy

PVR is a destructive complication of retinal detachment (RD) and vitreoretinal surgeries [40]. PVR is believed to represent a maladapted retinal wound healing process with proliferation of retinal and immune cells resulting in the formation of scar-like fibrous membranes which may cause tractional RD.

At present, surgical removal of the fibrous membranes and restoration of the physiological conditions are the first treatment option of PVR. Although the success rates of RD surgery was considerably improved by vitrectomy combined with silicone or C3F8 gas tamponade, the surgical treatment for PVR is often unsuccessful.

The development of PVR is a multifaceted process involving cellular and humoral factors. The results of earlier studies demonstrated that the cells that are critical for

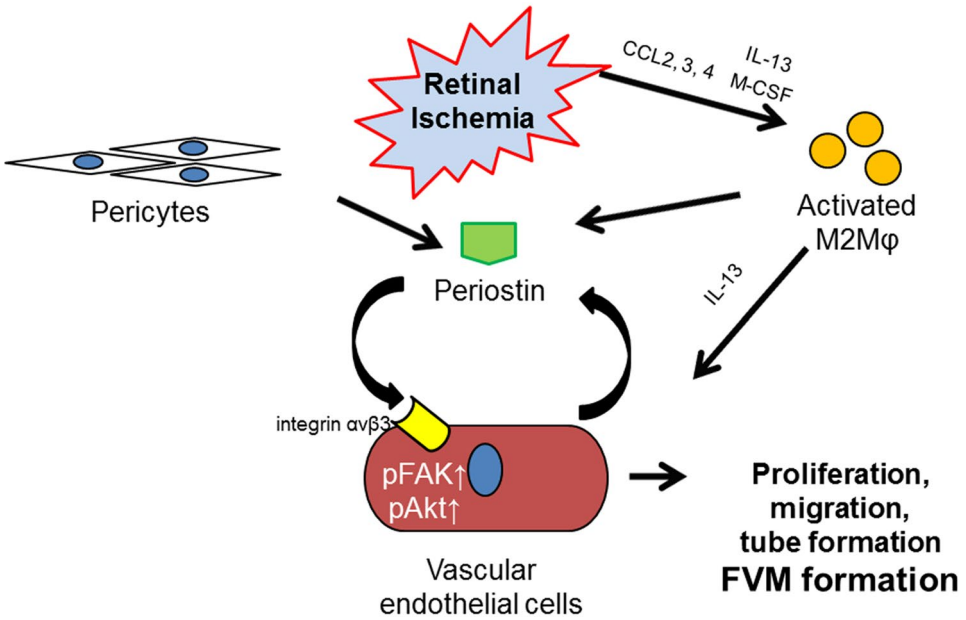

Fig. 2 Presumed mechanism of fibrovascular membrane formation induced by periostin-involving pathologic conditions in eyes with proliferative diabetic retinopathy (PDR). First, retinal ischemia may induce an upregulation in the expression of the CCL2, CCL3, and CCL4 genes which attract monocytes to the diabetic retina. Second, M-CSF released from diabetic retina transforms the recruited monocytes into M2-like macrophages. Third, IL-13 released from the Th2 cells in the retina further polarizes to activated M2 macrophages. Fourth, the polarized M2 macrophages and retinal pericytes produce periostin that promotes retinal neovascularization and fibrosis by Akt phosphorylation via integrin αvβ3. In parallel, the ischemia also stimulates the production of VEGF by retinal glial cells and vascular endothelial cells. These processes are likely to be important in promoting M2 macrophage-involved FVM formation in diabetic retinas

the formation of PVR-ERMs are glial cells, retinal pigment epithelial (RPE) cells, fibroblasts, and macrophages/monocytes [41].

Consistent with our global expression analysis [7], periostin mRNA expressions were detected in the PVR-ERMs, but were barely detectable in the normal retinas [42]. Vitreal concentrations of periostin in PVR patients were markedly elevated compared with those in patients with MH and RD [42]. The vitreal concentrations of TGFβ2 in PVR patients were also significantly higher than those in patients with MH and RD [42]. Additionally, there was a strong association between the vitreal concentrations of periostin and TGFβ2 in PVR patients. Moreover, Spearman's rank correlation showed that the vitreal concentrations of periostin were significantly correlated with PVR grade.

Immunohistochemical analysis exhibited elongated patterns of periostin expression in PVR-ERMs. In PVR-ERMs, RPE cells expressed periostin and α-SMA as well as integrin αV. These findings suggest that most of the myofibroblasts in the PVR-ERMs are transdifferentiated RPE cells and that periostin and/or integrin αV are predominantly expressed in the RPE cells but not in the glial cells.

In vitro, periostin increased proliferation, migration, adhesion, and collagen production in RPE cells via integrin αV-mediated FAK and AKT phosphorylation [42]. Periostin inhibition suppressed migration and adhesion induced by PVR vitreous and TGFβ2. In vivo, periostin blockade had the inhibitory effect on progression of rabbit experimental PVR without affecting the viability of retinal cells [42].

Although the etiology of PVR is not fully understood, there is considerable evidence that a variety of cytokines and growth factors present in the vitreous regulate the fibrous membrane formation [43–47]. Those factors promote cellular responses indispensable for PVR, including cell proliferation, adhesion and migration [43–47]. In a recent study, a cocktail of reagents neutralizing eight cytokines or growth factors, including transforming growth factor-βs (TGF-βs), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and PDGF receptor α (PDGFRα), inhibited experimental PVR [48]. In contrast, blocking solely periostin showed an equivalent inhibitory effect on PVR progression. Moreover, periostin blockade alone inhibited PVR vitreous-induced cell migration and adhesion, in spite of the presence of all the other factors in the vitreous. This may be because periostin expression is regulated by those growth factors or their receptors [49, 50]. Therefore, blockade of periostin predominates the deleterious effects of the upstream PVR-driving growth factors.

These results identified periostin as an important molecule for fibrous membrane formation (Fig. 3) and a promising therapeutic target for PVR.

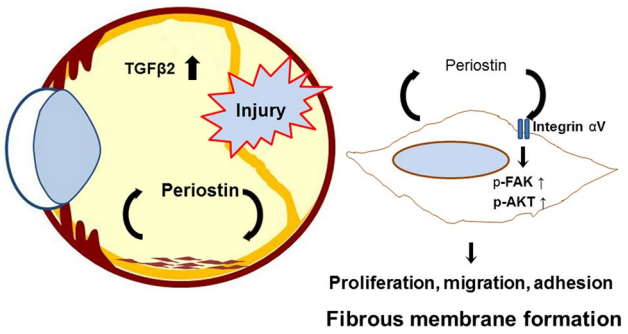

Fig. 3 Presumed mechanism of fibrous membrane formation induced by periostin-involving pathologic conditions in eyes with proliferative vitreoretinopathy (PVR). Ocular injury causes the enhancement of TGFβ2 production and dispersion of RPE cells onto the retina. TGFβ2 induces trans-differentiation of RPE cells into myofibroblasts resulting in periostin production. Periostin acts in an autocrine fashion to stimulate FAK and AKT phosphorylation via the αV integrin, promoting cell proliferation, adhesion and migration, etc., leading to the fibrous membrane formation

Periostin in age-related macular degeneration

Age-related macular degeneration (AMD) is a leading cause of a severe vision loss in the older population of developed countries [51]. It is estimated that the prevalence of AMD will increase, which would then accelerate both the medical and social burdens of the countries. At the advanced stage of AMD, choroidal FVMs, which are made up of choroidal neovascularization (CNV) and choroidal fibrosis, can lead to severe vision loss [52]. In this process, there is proliferation, migration and adhesion of various types of cells, including vascular endothelial cells, RPE cells, fibroblasts, glial cells and macrophages/monocytes. There is also deposition of matricellular proteins [53]. Several growth factors, such as VEGFs, placental growth factor, tenascin-C, connective tissue growth factor and TGF-βs and their receptors, are involved in this process [54, 55].

To examine whether periostin is involved in the formation of choroidal FVMs, the expression of the periostin mRNA in the RPE–choroid complexes after laser injury was compared with that in normal RPE–choroid complexes in a mouse model of laser-induced CNV. The expression of periostin mRNA in a mouse CNV model group was significantly higher compared with the control group and reached a peak on day 14 [3]. Immunohistochemical analyses exhibited periostin-positive staining in RPE65-positive RPE cells after the laser injury. In the human choroidal FVMs, periostin was enhanced in the cytokeratin-positive RPE cells [3]. These findings suggested that the periostin expression was enhanced in the RPE cells both in the FVMs of AMD patients and in mouse CNV model.

To further examine whether periostin enhances the formation of choroidal FVMs, we quantified the volume of the

CNVs at day 7 and fibrous volumes at day 21 in both periostin KO and WT mice. The average CNV volumes in the periostin KO mice group were significantly smaller than that of WT mice group. There was an approximately 60% reduction in the average fibrosis volume in periostin KO mice than in the WT mice group [3]. These findings suggest that periostin is a promoter of choroidal FVM formation.

Development of innovative periostin-targeting ribonucleic acid drugs

RNA interference (RNAi) is a natural mechanism of post-transcriptional silencing of gene expression that has been recently considered to be a novel type of therapeutic system [56]. Because of their high potency and selectivity, RNAi-based therapy has several advantages over conventional therapeutic options including antisense, antibody and aptamer therapy. Moreover, RNAi agents can be easily synthesized, and the processes required for identifying and optimizing them are prompt. However, previous investigations using canonical double-stranded small interfering RNAs (siRNAs) revealed several obstacles such as the adverse off-target effects through Toll-like receptor 3 (TLR3) activation, the lack of a safe drug delivery system (DDS), and the lack of stability [57–60]. We developed a novel single-stranded RNAi agent, NK0144, targeting periostin that self-anneals into a distinctive structure containing a canonical double-stranded RNA to overcome these obstacles (Fig. 4) [25].

In vivo inhibitory effect of single-stranded RNAi agent targeting periostin on retinal neovascularization

We have demonstrated that this single-stranded RNAi agent which targets periostin (NK0144) significantly inhibits the migration and tube formation of HRECs driven by IL-13, and the preretinal pathological NV in OIR retinas by an intravitreal injection without any DDS [25]. In addition, the inhibitory effect of the single-stranded RNAi agent was larger than the canonical double-stranded siRNA (NI0079). Moreover, treatment with NK0144 resulted in a significant increase in the physiological revascularization compared to the treatment control.

The sequence used for periostin knockdown exists not only in human periostin but also in mouse, rat, rabbit, and rhesus macaque periostin [3]. This suggests that NK0144 can be utilized for both in vitro and in vivo experiments and would also be suitable for clinical trials in the future. The mechanisms that determine the differences of the effect on ischemia-induced retinal NV between the single-stranded RNAi and the canonical double-stranded siRNA agent were not completely demonstrated. However, we assume that these are because the single-stranded RNAi agent has no off-target gene silencing, better stability against nuclease, and no immunostimulatory effects via TLR3 activation [3, 61–63]. Therefore, intravitreal injection of naked single-stranded RNAi agent targeting periostin may be a safer and a more efficient therapeutic strategy for blocking preretinal pathological NV.

In vivo inhibitory effect of single-stranded RNAi agent targeting periostin on progression of choroidal FVM formation

We have also demonstrated that naked NK0144 significantly inhibits the expression of periostin, proliferation, adhesion and migration of RPE cells without influence on cell viability [3]. Moreover, we observed that labeled single-stranded RNA without any DDS was detectable in the RPE-choroid for at least 5 days after an intravitreal injection [3]. This indicates that it was retained within cells at the CNV site for a considerable period of time. In contrast to canonical double-stranded siRNAs, we found that naked NK0144 significantly inhibited choroidal FVM formation (both NV and fibrosis) without serious toxicity.

These results strongly suggest that intravitreal injections of naked NK0144 may also be a safer and more efficient therapeutic option to inhibit choroidal FVM.

Although anti-VEGF therapy for PDR and AMD is now a mainstream therapy to prevent retinal and choroidal FVM

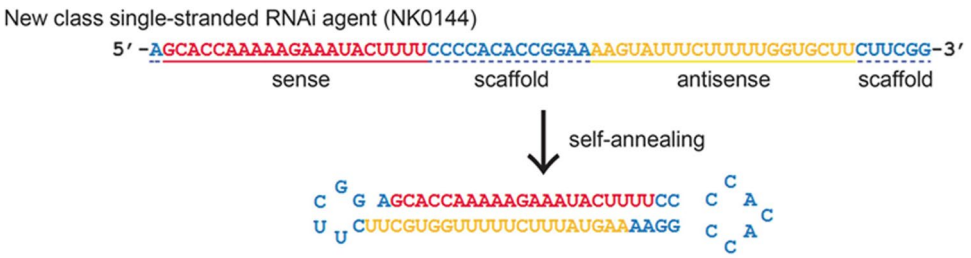

Fig. 4 Structure of novel class of single-stranded RNAi agent. Novel class of RNAi agent was prepared as single-stranded RNA oligomers that self-anneal as shown. Nucleotides in red indicate the sense strand of the target (periostin), nucleotides in yellow are the antisense strand, and nucleotides in blue are the scaffold

formation, it was recently reported that anti-VEGF therapy may cause impairment of the normal retinal function and the maintenance of the choriocapillaris [64]. This is partly because VEGF plays a pivotal role in retinal homeostasis. Therefore, therapies that block VEGF to inhibit pathological NV could result in unexpected complications of the normal retina and should be used carefully. In contrast to VEGF, we have demonstrated that periostin was barely detected in the normal retina [7, 24]. We also reported that the correlation between the vitreous concentration of VEGF and periostin was weak in PDR patients [24]. Additionally, previous studies have demonstrated that the binding of VEGF with VEGF receptor-2 (VEGFR2) promoted NV mainly through the PLCγ/PKC/MAPK pathway [65], whereas the binding of periostin with integrin αvβ3 promotes NV mainly via the FAK/Akt pathway [25, 42]. These are good evidences of the concept that anti-periostin therapy may have independent effects on retinal and choroidal FVM formation from anti-VEGF therapy. Therefore, periostin may be an interesting therapeutic target to regulate "disease-specific" pathways involved in the formation of retinal and choroidal FVM, while minimizing the unfavorable side effects on the normal retina. Additional preclinical studies regarding the stability, toxicity and effect of duration are underway for establishing the novel periostin-targeting RNAi agent for combating retinal and choroidal FVM formation.

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References

1. Yoshida S (2014) Identification of molecular targets for intraocular proliferative diseases using genomicapproaches. J Jpn Ophthalmol Soc 118:241–282
2. Hiscott P, Wong D, Grierson I (2000) Challenges in ophthalmic pathology: the vitreoretinal membrane biopsy. Eye 14(Pt 4):549–559
3. Nakama T, Yoshida S, Ishikawa K, Kobayashi Y, Zhou Y, Nakao S, Sassa Y, Oshima Y, Takao K, Shimahara A, Yoshikawa K, Hamasaki T, Ohgi T, Hayashi H, Matsuda A, Kudo A, Nozaki M, Ogura Y, Kuroda M, Ishibashi T (2015) Inhibition of choroidal fibrovascular membrane formation by new class of rna interference therapeutic agent targeting periostin. Gene Ther 22:127–137
4. Kirchhof B (2004) Strategies to influence PVR development. Graefes Arch Clin Exp Ophthalmol 242:699–703
5. Kobayashi Y, Yoshida S, Zhou Y, Nakama T, Ishikawa K, Arima M, Nakao S, Sassa Y, Takeda A, Hisatomi T, Ikeda Y, Matsuda A, Sonoda KH, Ishibashi T (2016) Tenascin-c promotes angiogenesis in fibrovascular membranes in eyes with proliferative diabetic retinopathy. Mol Vis 22:436–445
6. Yoshida S, Ogura A, Ishikawa K, Yoshida A, Kohno R, Yamaji Y, Ikeo K, Gojobori T, Kono T, Ishibashi T (2010) Gene expression profile of fibrovascular membranes from patients with proliferative diabetic retinopathy. Br J Ophthalmol 94:795–801
7. Ishikawa K, Yoshida S, Kobayashi Y, Zhou Y, Nakama T, Nakao S, Sassa Y, Oshima Y, Niiro H, Akashi K, Kono T, Ishibashi T (2015) Microarray analysis of gene expression in fibrovascular membranes excised from patients with proliferative diabetic retinopathy. Investig Ophthalmol Vis Sci 56:932–946
8. Asato R, Yoshida S, Ogura A, Nakama T, Ishikawa K, Nakao S, Sassa Y, Enaida H, Oshima Y, Ikeo K, Gojobori T, Kono T, Ishibashi T (2013) Comparison of gene expression profile of epiretinal membranes obtained from eyes with proliferative vitreoretinopathy to that of secondary epiretinal membranes. PLoS One 8:e54191
9. Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, Rogers R, Chen Z, Maeda M, Kruzynska-Frejtag A, Feng JQ, Conway SJ (2005) Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Mol Cell Biol 25:11131–11144
10. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, Li F, Ingram DA, Menick D, Field L, Firulli AB, Molkentin JD, Markwald R, Conway SJ (2008) Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. Circ Res 102:752–760
11. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J (2011) Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 481:85–89
12. Shimazaki M, Nakamura K, Kii I, Kashima T, Amizuka N, Li M, Saito M, Fukuda K, Nishiyama T, Kitajima S, Saga Y, Fukayama M, Sata M, Kudo A (2008) Periostin is essential for cardiac healing after acute myocardial infarction. J Exp Med 205:295–303
13. Conway SJ, Molkentin JD (2008) Periostin as a heterofunctional regulator of cardiac development and disease. Curr Genomics 9:548–555
14. Ontsuka K, Kotobuki Y, Shiraishi H, Serada S, Ohta S, Tanemura A, Yang L, Fujimoto M, Arima K, Suzuki S, Murota H, Toda S, Kudo A, Conway SJ, Narisawa Y, Katayama I, Izuhara K, Naka T (2012) Periostin, a matricellular protein, accelerates cutaneous wound repair by activating dermal fibroblasts. Exp Dermatol 21:331–336
15. Masuoka M, Shiraishi H, Ohta S, Suzuki S, Arima K, Aoki S, Toda S, Inagaki N, Kurihara Y, Hayashida S, Takeuchi S, Koike K, Ono J, Noshiro H, Furue M, Conway SJ, Narisawa Y, Izuhara K (2012) Periostin promotes chronic allergic inflammation in response to Th2 cytokines. J Clin Investig 122:2590–2600
16. Sivaprasad S, Gupta B, Crosby-Nwaobi R, Evans J (2012) Prevalence of diabetic retinopathy in various ethnic groups: a worldwide perspective. Surv Ophthalmol 57:347–370
17. Yoshida A, Yoshida S, Ishibashi T, Inomata H (1999) Intraocular neovascularization. Histol Histopathol 14:1287–1294
18. Yoshida S, Kubo Y, Kobayashi Y, Zhou Y, Nakama T, Yamaguchi M, Tachibana T, Ishikawa K, Arita R, Nakao S, Sassa Y, Oshima Y, Kono T, Ishibashi T (2015) Increased vitreous concentrations of MCP-1 and IL-6 after vitrectomy in patients with proliferative diabetic retinopathy: possible association with postoperative macular oedema. Br J Ophthalmol 99:960–966
19. Yoshida S, Nakama T, Ishikawa K, Arima M, Tachibana T, Nakao S, Sassa Y, Yasuda M, Enaida H, Oshima Y, Kono T, Ishibashi T (2012) Antiangiogenic shift in vitreous after vitrectomy in patients with proliferative diabetic retinopathy. Investig Ophthalmol Vis Sci 53:6997–7003
20. Tachibana T, Yoshida S, Kubo Y, Koayashi Y, Nakama T, Ishikawa K, Nakao S, Izuhara K, Kono T, Ishibashi T (2016) Reduced vitreal concentration of periostin after vitrectomy in patients with proliferative diabetic retinopathy. Acta Ophthalmol 94:e81–e82

21. Yoshida S, Ishikawa K, Matsumoto T, Yoshida A, Ishibashi T, Kono T (2010) Reduced concentrations of angiogenesis-related factors in vitreous after vitrectomy in patients with proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 248:799–804
22. Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C (2006) Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr Diabetes Rev 2:71–98
23. Nakama T, Yoshida S, Ishikawa K, Kobayashi Y, Abe T, Kiyonari H, Shioi G, Katsuragi N, Ishibashi T, Morishita R, Taniyama Y (2016) Different roles played by periostin splice variants in retinal neovascularization. Exp Eye Res 153:133–140
24. Yoshida S, Ishikawa K, Asato R, Arima M, Sassa Y, Yoshida A, Yoshikawa H, Narukawa K, Obika S, Ono J, Ohta S, Izuhara K, Kono T, Ishibashi T (2011) Increased expression of periostin in vitreous and fibrovascular membranes obtained from patients with proliferative diabetic retinopathy. Investig Ophthalmol Vis Sci 52:5670–5678
25. Nakama T, Yoshida S, Ishikawa K, Kubo Y, Kobayashi Y, Zhou Y, Nakao S, Hisatomi T, Ikeda Y, Takao K, Yoshikawa K, Matsuda A, Ono J, Ohta S, Izuhara K, Kudo A, Sonoda KH, Ishibashi T (2017) Therapeutic effect of novel single-stranded RNAi agent targeting periostin in eyes with retinal neovascularization. Mol Ther Nucleic Acids 6:279–289
26. Ishikawa K, Yoshida S, Nakao S, Sassa Y, Asato R, Kohno R, Arima M, Kita T, Yoshida A, Ohuchida K, Ishibashi T (2012) Bone marrow-derived monocyte lineage cells recruited by MIP-1beta promote physiological revascularization in mouse model of oxygen-induced retinopathy. Lab Investig 92:91–101
27. Snead DR, James S, Snead MP (2008) Pathological changes in the vitreoretinal junction 1: epiretinal membrane formation. Eye 22:1310–1317
28. Sunderkotter C, Beil W, Roth J, Sorg C (1991) Cellular events associated with inflammatory angiogenesis in the mouse cornea. Am J Pathol 138:931–939
29. Yoshida S, Yoshida A, Ishibashi T, Elner SG, Elner VM (2003) Role of MCP-1 and MIP-1alpha in retinal neovascularization during postischemic inflammation in a mouse model of retinal neovascularization. J Leukoc Biol 73:137–144
30. Ishikawa K, Yoshida S, Kadota K, Nakamura T, Niiro H, Arakawa S, Yoshida A, Akashi K, Ishibashi T (2010) Gene expression profile of hyperoxic and hypoxic retinas in a mouse model of oxygen-induced retinopathy. Investig Ophthalmol Vis Sci 51:4307–4319
31. Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483
32. Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. J Clin Investig 122:787–795
33. Kobayashi Y, Yoshida S, Nakama T, Zhou Y, Ishikawa K, Arita R, Nakao S, Miyazaki M, Sassa Y, Oshima Y, Izuhara K, Kono T, Ishibashi T (2015) Overexpression of CD163 in vitreous and fibrovascular membranes of patients with proliferative diabetic retinopathy: possible involvement of periostin. Br J Ophthalmol 99:451–456
34. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M (2013) Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229:176–185
35. Yoshida S, Kobayashi Y, Nakama T, Zhou Y, Ishikawa K, Arita R, Nakao S, Miyazaki M, Sassa Y, Oshima Y, Izuhara K, Kono T, Ishibashi T (2015) Increased expression of M-CSF and IL-13 in vitreous of patients with proliferative diabetic retinopathy: implications for M2 macrophage-involving fibrovascular membrane formation. Br J Ophthalmol 99:629–634
36. Liu W, Xu GZ, Jiang CH, Da CD (2009) Expression of macrophage colony-stimulating factor (M-CSF) and its receptor in streptozotocin-induced diabetic rats. Curr Eye Res 34:123–133
37. Zhou Y, Yoshida S, Nakao S, Yoshimura T, Kobayashi Y, Nakama T, Kubo Y, Miyawaki K, Yamaguchi M, Ishikawa K, Oshima Y, Akashi K, Ishibashi T (2015) M2 macrophages enhance pathological neovascularization in the mouse model of oxygen-induced retinopathy. Investig Ophthalmol Vis Sci 56:4767–4777
38. Zurawski SM, Vega F Jr, Huyghe B, Zurawski G (1993) Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J 12:2663–2670
39. O'Reilly S (2013) Role of interleukin-13 in fibrosis, particularly systemic sclerosis. Biofactors 39:593–596
40. Leiderman YI, Miller JW (2009) Proliferative vitreoretinopathy: pathobiology and therapeutic targets. Semin Ophthalmol 24:62–69
41. Hiscott PS, Grierson I, McLeod D (1984) Retinal pigment epithelial cells in epiretinal membranes: an immunohistochemical study. Br J Ophthalmol 68:708–715
42. Ishikawa K, Yoshida S, Nakao S, Nakama T, Kita T, Asato R, Sassa Y, Arita R, Miyazaki M, Enaida H, Oshima Y, Murakami N, Niiro H, Ono J, Matsuda A, Goto Y, Akashi K, Izuhara K, Kudo A, Kono T, Hafezi-Moghadam A, Ishibashi T (2014) Periostin promotes the generation of fibrous membranes in proliferative vitreoretinopathy. FASEB J 28:131–142
43. Kita T, Hata Y, Arita R, Kawahara S, Miura M, Nakao S, Mochizuki Y, Enaida H, Goto Y, Shimokawa H, Hafezi-Moghadam A, Ishibashi T (2008) Role of TGF-beta in proliferative vitreoretinal diseases and rock as a therapeutic target. Proc Natl Acad Sci USA 105:17504–17509
44. Banerjee S, Savant V, Scott RA, Curnow SJ, Wallace GR, Murray PI (2007) Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. Investig Ophthalmol Vis Sci 48:2203–2207
45. Harada C, Mitamura Y, Harada T (2006) The role of cytokines and trophic factors in epiretinal membranes: involvement of signal transduction in glial cells. Prog Retin Eye Res 25:149–164
46. He S, Chen Y, Khankan R, Barron E, Burton R, Zhu D, Ryan SJ, Oliver N, Hinton DR (2008) Connective tissue growth factor as a mediator of intraocular fibrosis. Investig Ophthalmol Vis Sci 49:4078–4088
47. Elner SG, Elner VM, Jaffe GJ, Stuart A, Kunkel SL, Strieter RM (1995) Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Curr Eye Res 14:1045–1053
48. Pennock S, Rheaume MA, Mukai S, Kazlauskas A (2011) A novel strategy to develop therapeutic approaches to prevent proliferative vitreoretinopathy. Am J Pathol 179:2931–2940
49. Li G, Oparil S, Sanders JM, Zhang L, Dai M, Chen LB, Conway SJ, McNamara CA, Sarembock IJ (2006) Phosphatidylinositol-3-kinase signaling mediates vascular smooth muscle cell expression of periostin in vivo and in vitro. Atherosclerosis 188:292–300
50. Dangaria SJ, Ito Y, Walker C, Druzinsky R, Luan X, Diekwisch TG (2009) Extracellular matrix-mediated differentiation of periodontal progenitor cells. Differentiation 78:79–90
51. de Jong PT (2006) Age-related macular degeneration. N Engl J Med 355:1474–1485
52. Schlingemann RO (2004) Role of growth factors and the wound healing response in age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 242:91–101
53. Bloch SB, Lund-Andersen H, Sander B, Larsen M (2013) Subfoveal fibrosis in eyes with neovascular age-related macular degeneration treated with intravitreal ranibizumab. Am J Ophthalmol 156(116–124):e111
54. Daniel E, Toth CA, Grunwald JE, Jaffe GJ, Martin DF, Fine SL, Huang J, Ying GS, Hagstrom SA, Winter K, Maguire MG, Comparison of Age-related Macular Degeneration Treatments Trials Research G (2014) Risk of scar in the comparison of

age-related macular degeneration treatments trials. Ophthalmology 121:656–666
55. Kobayashi Y, Yoshida S, Zhou Y, Nakama T, Ishikawa K, Kubo Y, Arima M, Nakao S, Hisatomi T, Ikeda Y, Matsuda A, Sonoda KH, Ishibashi T (2016) Tenascin-c secreted by transdifferentiated retinal pigment epithelial cells promotes choroidal neovascularization via integrin alphav. Lab Investig 96:1178–1188
56. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide rnas mediate rna interference in cultured mammalian cells. Nature 411:494–498
57. Pecot CV, Calin GA, Coleman RL, Lopez-Berestein G, Sood AK (2011) Rna interference in the clinic: challenges and future directions. Nat Rev Cancer 11:59–67
58. Kleinman ME, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, Albuquerque RJ, Yamasaki S, Itaya M, Pan Y, Appukuttan B, Gibbs D, Yang Z, Kariko K, Ambati BK, Wilgus TA, DiPietro LA, Sakurai E, Zhang K, Smith JR, Taylor EW, Ambati J (2008) Sequence- and target-independent angiogenesis suppression by sirna via tlr3. Nature 452:591–597
59. Yang Z, Stratton C, Francis PJ, Kleinman ME, Tan PL, Gibbs D, Tong Z, Chen H, Constantine R, Yang X, Chen Y, Zeng J, Davey L, Ma X, Hau VS, Wang C, Harmon J, Buehler J, Pearson E, Patel S, Kaminoh Y, Watkins S, Luo L, Zabriskie NA, Bernstein PS, Cho W, Schwager A, Hinton DR, Klein ML, Hamon SC, Simmons E, Yu B, Campochiaro B, Sunness JS, Campochiaro P, Jorde L, Parmigiani G, Zack DJ, Katsanis N, Ambati J, Zhang K (2008) Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. N Engl J Med 359:1456–1463
60. Cho WG, Albuquerque RJ, Kleinman ME, Tarallo V, Greco A, Nozaki M, Green MG, Baffi JZ, Ambati BK, De Falco M, Alexander JS, Brunetti A, De Falco S, Ambati J (2009) Small interfering RNA-induced TLR3 activation inhibits blood and lymphatic vessel growth. Proc Natl Acad Sci USA 106:7137–7142
61. Hamasaki T, Suzuki H, Shirohzu H, Matsumoto T, D'Alessandro-Gabazza CN, Gil-Bernabe P, Boveda-Ruiz D, Naito M, Kobayashi T, Toda M, Mizutani T, Taguchi O, Morser J, Eguchi Y, Kuroda M, Ochiya T, Hayashi H, Gabazza EC, Ohgi T (2012) Efficacy of a novel class of rna interference therapeutic agents. PLoS One 7:e42655
62. Fujita Y, Takeshita F, Mizutani T, Ohgi T, Kuwano K, Ochiya T (2013) A novel platform to enable inhaled naked RNAi medicine for lung cancer. Sci Rep 3:3325
63. Takanashi M, Sudo K, Ueda S, Ohno S, Yamada Y, Osakabe Y, Goto H, Matsunaga Y, Ishikawa A, Usui Y, Kuroda M (2015) Novel types of small RNA exhibit sequence- and target-dependent angiogenesis suppression without activation of toll-like receptor 3 in an age-related macular degeneration (AMD) mouse model. Mol Ther Nucleic Acids 4:e258
64. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA (2009) An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. Proc Natl Acad Sci USA 106:18751–18756
65. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L (2006) Vegf receptor signalling—in control of vascular function. Nat Rev Mol Cell Biol 7:359–371