Antagonizing Wnt Pathway in Diabetic Retinopathy

Ling Zhao,[1,2] Sherrina H. Patel,[2] Jacqueline Pei,[1,2] and Kang Zhang[1,2,3]

In this issue, Liu et al. (1) reported that kallistatin, an endogenous Wnt antagonist, exerted antiangiogenic and antineuroinflammatory effects by inhibiting canonical Wnt signaling in diabetic retinopathy (DR) mouse models.

DR is one of the most common microvascular complications of diabetes, characterized by retinal vascular leakage, inflammation, and abnormal neovascularization (2,3). DR has become the most frequent cause of blindness and loss of visual acuity among working adults in developed countries, and the incidence continues to rise (4). At present, laser photocoagulation and antivascular endothelial growth factor (anti-VEGF) therapies are standard-of-care treatment options. However, laser photocoagulation therapy only targets advanced stages of disease (5). Although anti-VEGF therapy can significantly inhibit vascular permeability and slow the progression of DR, recent clinical trials have showed that it is not effective for all patients and that some patients develop diminished responses to therapy after long-term use. Many patients can only benefit from a partial response to treatment. Additionally, anti-VEGF therapy results in some adverse events (6). Due to the limitations of current treatments, new strategies for DR therapy are urgently needed.

The pathology of DR is complex. A better understanding of the causative underlying mechanisms of DR is very important to find novel treatments. In addition to VEGF, a number of proangiogenic signaling pathways as well as inflammatory cytokines are linked to DR pathogenesis (6). Accumulating evidences have shown that the Wnt signaling pathway plays a fundamental role in multiple physiological and pathological processes, including angiogenesis and inflammation (7–10). Loss or gain of function of Wnt pathway components causes abnormal vascular development and angiogenesis (7,11). Mutations in *FZD4*, *LRP5*, and *NDP* result in severely defective retinal vascularization (7,12,13).

Wnt pathways can be broadly divided into two types, canonical and noncanonical. The canonical Wnt pathway is mediated by the transcriptional activity of β-catenin (Fig. 1*A*). Activation of the canonical pathway is initiated when Wnt binds to the coreceptor complex of Frizzled (Fz) and low-density lipoprotein receptor-related protein (LRP5/6). Subsequent events lead to the inactivation of glycogen synthase kinase-3β (GSK3β), preventing phosphorylation of β-catenin. Thus, β-catenin is blocked from degradation and accumulates in the cytosol. β-catenin then translocates to the nucleus and forms a complex with T-cell factor/lymphoid enhancer factor (TCF4/LEF), to promote the expression of Wnt target genes including VEGF (8,14). The canonical β-catenin–dependent Wnt pathway plays a central role in development and differentiation of microvasculature in retina and brain (7,13). Overactivation of canonical Wnt signaling can promote pathological processes in DR. Nuclear β-catenin accumulation has been observed in the retinas of three DR animal models and in human patients with DR. Furthermore, the expression of LRP5/6 was upregulated in the retinas of DR models (14). Another study provided genetic evidence that TCF7L2 (TCF4) was found to be not only associated with type 2 diabetes, but also with proliferative DR (PDR) in Caucasian patients with type 2 diabetes (15). In the retinas of one DR model, the expression of TCF7L2 was found to be significantly higher compared with normal controls (15). Intravitreal injection of DKK1, a Wnt-signaling antagonist that blocks the dimerization of LRP5/6 with Fz receptor, is sufficient to ameliorate DR (14). These studies showed that aberrant activation of Wnt signaling plays a causative role in the progression of DR.

Owing to the significance of Wnt signaling in angiogenesis, Wnt antagonists have been considered potential treatments for neovascular disorders (11). SERPINA3K is an endogenous Wnt antagonist that can bind to LRP6 and inhibit vascular permeability and inflammation in diabetic rats (16). Pigment epithelium–derived factor acts as an endogenous anti-inflammatory factor preventing vascular hyperpermeability in diabetes and oxygen-induced retinopathy (OIR) rat models (17). It is an inhibitor of the canonical Wnt pathway by binding LRP6 and preventing LRP6–Fz receptor dimerization and signaling (10). Inhibiting Wnt signaling with a monoclonal antibody that binds to LRP6 also protects against vascular leakage and inflammation in the retinas of diabetic animals (18). Through inhibition of aberrant Wnt signaling, antagonists of Wnt pathway have become potential therapies in DR.

Kallistatin is a member of the serine proteinase inhibitor superfamily that inhibits angiogenesis, inflammation, tumor growth, and metastasis in animal models and in cultured cells (19,20). It is present not only in the plasma, but also in various tissues, cells, and bodily fluids (21). A previous study found that kallistatin is produced endogenously in the eye and that vitreous levels of kallistatin were significantly reduced in patients with DR, suggesting that kallistatin might have a protective effect on microvasculature (19). To investigate the functions and mechanism of kallistatin in DR, Liu et al. (1) generated kallistatin-transgenic (kallistatin-TG) mice that express physiologically high levels of kallistatin in the retina. The retinas of kallistatin-TG mice have normal histological structure and functions. The OIR mouse model has been used extensively to study ischemia-induced retinopathy (22). Compared with wild-type mice with OIR, ischemia-induced angiogenesis was

From the [1]Molecular Medicine Research Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan, China; the [2]Department of Ophthalmology and Shiley Eye Center, and Institute for Genomic Medicine, University of California, San Diego, La Jolla, California; and the [3]Veterans Administration Healthcare System, San Diego, California.
Corresponding author: Kang Zhang, kang.zhang@gmail.com.
DOI: 10.2337/db13-1309

See accompanying original article, p. 4228.

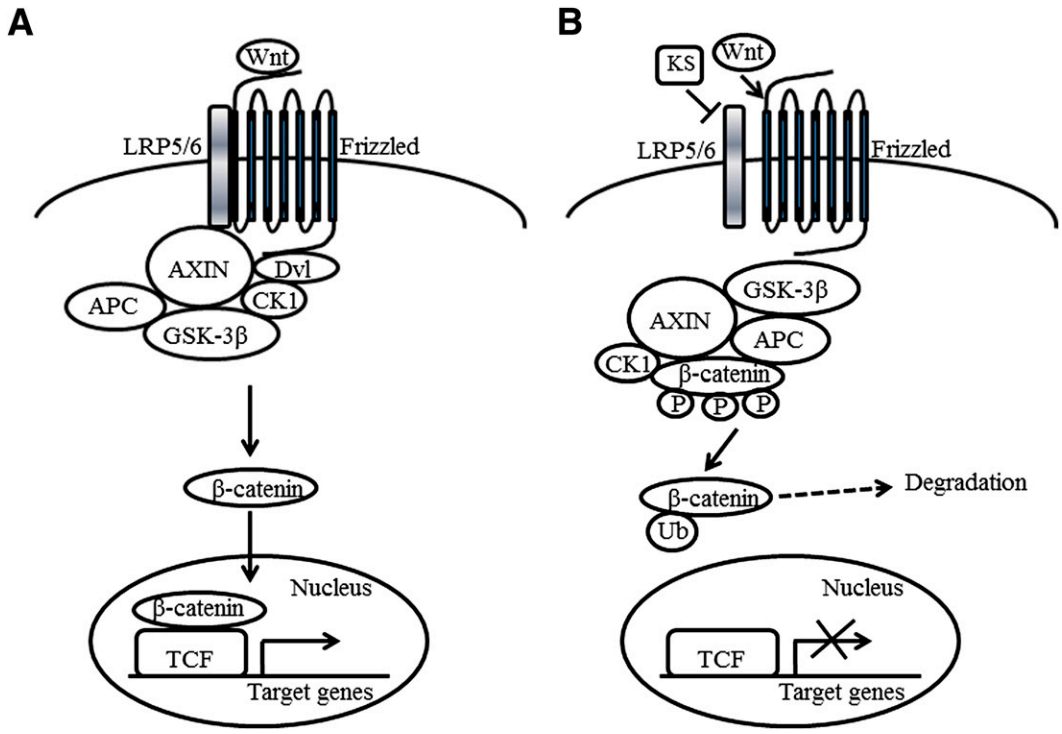

FIG. 1. A schematic view of kallistatin action in the canonical Wnt pathway. *A*: The canonical Wnt pathway is activated when Wnt binds to coreceptor complex of Frizzled and LRP5/6. Then GSK-3β is inactivated by the action of a multiplex including AXIN and Dvl. Phosphorylation and degradation of β-catenin is inhibited, which leads to β-catenin accumulation in the cytoplasm and its translocation to the nucleus. In the nucleus, β-catenin binds and complexes with TCF4/LEF to activate transcription of target genes. *B*: Kallistatin inhibits Wnt signaling by binding to LRP6. Cytosolic β-catenin is phosphorylated by the complex containing AXIN/APC/CK1/GSK-3β. Phosphorylated β-catenin is ubiquitinated and degraded in the cytoplasm. KS, kallistatin; APC, adenomatous polyposis coli; Dvl, Dishevelled; CK1, casein kinase 1; P, phosphorylation; Ub, ubiquitination.

less severe in kallistatin-TG mice with OIR. Preretinal neovascularization and retinal proinflammatory cytokines, VEGF and intracellular adhesion molecule 1 (ICAM-1), were found to be reduced in kallistatin-TG mice with OIR. Liu et al. (1) also induced diabetes in kallistatin-TG mice by crossing kallistatin-TG with Akita mice (a genetic model of type 1 diabetes). Inhibition of retinal neuroinflammation and vascular leakage was observed in Akita × kallistatin-TG mice. In Akita × kallistatin-TG models, CD11b monocytes were found to be the major cell type involved in leukostasis, and the reduction of adherent $CD11b^+$ leukocytes was due to downregulation of the endothelial ICAM-1 level by kallistatin. Liu et al. (1) also studied a diabetic BAT-gal reporter × kallistatin-TG model induced by streptozocin, providing direct evidence of diabetes-induced Wnt signaling in the retina and the suppression of this Wnt signaling by kallistatin. Furthermore, they identified the mechanism of kallistatin as an endogenous antagonist of Wnt signaling in diabetic conditions or ischemia. Kallistatin blocks the canonical Wnt pathway by specifically binding to the extracellular domain of LRP6 with high affinity and competing with Wnt3a. Thus, kallistatin inhibits the transcriptional activity of β-catenin induced by Wnt ligand (Fig. 1*B*).

Liu et al. (1) contribute to a better understanding of the roles of kallistatin in DR, as well as the effects and mechanism of kallistatin in vivo at reasonable physiological levels. These results provide key findings that will advance future studies on Wnt antagonists. In summary, antagonists of Wnt pathway exert antiangiogenic and anti-inflammatory activities in DR and therefore represent an attractive potential therapy for the treatment of DR and other diseases involving pathological neovascularization.

ACKNOWLEDGMENTS

This work is supported in part by grants from the 973 Program (2013CB967504), the KACST UCSD Center of Excellence in Nanomedicine, and the Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

No potential conflicts of interest relevant to this article were reported.

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