Galectin-1, an angiogenic factor associated with diabetic retinopathy, is regulated by advanced glycation end products triggering inflammatory cues

Atsuhiro Kanda, Yoko Dong, Kousuke Noda, Susumu Ishida
Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Japan
Financial interests: none

Background: Diabetic retinopathy (DR) is the most common microvascular complication in patients with diabetes, and may have a debilitating impact on visual acuity, eventually leading to blindness.

Galectins, an evolutionarily conserved family of galactoside-binding lectin proteins, bind to cell surface glycol-conjugated proteins or lipids, and regulate a myriad of biological responses without having specific receptors like cytokines do. Galectin-1, encoded by the *LGALS1* gene, contributes to cell adhesion/proliferation and immunosuppression in a variety of cancer cells and regulatory T lymphocytes, respectively. Recently, we and others have revealed that galectin-1 interacts with the *N*-glycans of VEGFR2, enhancing phosphorylation of VEGFR2 and activating its downstream signal transduction in endothelial cells, so as to promote angiogenesis (1, 2). Importantly, vitreous aspirates from eyes with proliferative DR (PDR) showed higher protein levels of galectin-1 than those from non-diabetic controls (2). Moreover, the elevated levels of galectin-1 were not correlated with VEGF levels also increased in PDR eyes, suggesting that these two pro-angiogenic molecules were independently regulated (2).

In this study, we investigated protein levels of galectin-1 in eyes with the different clinical stages of DR, and explored upstream regulatory stimuli for galectin-1 expression selectively in the pathogenesis of DR.

Patients & Methods: Human surgical samples were examined by ELISA and immunofluorescence. Real-time PCR were performed to measure mRNA expression levels in human cell lines.

Results:

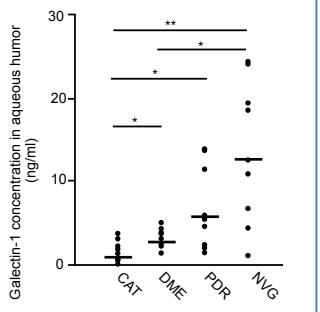

Fig. 1. Elevation of galectin-1 protein levels in aqueous humor samples collected from DR

Galectin-1 protein levels in aqueous humors increased along with the progression of clinical stages of diabetic retinopathy (DME, diabetic macular edema; PDR, proliferative DR; NVG, neovascular glaucoma) compared with CAT (age-related cataract).

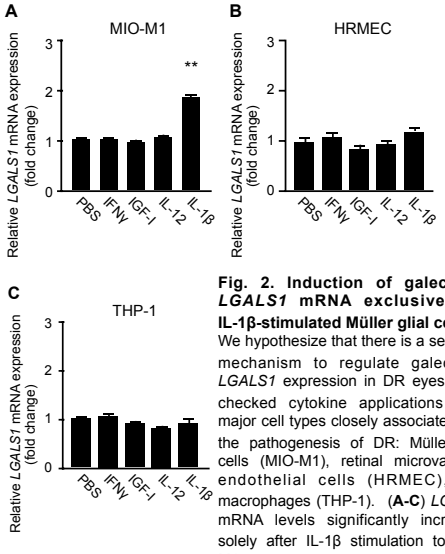

Fig. 2. Induction of galectin-1/*LGALS1* mRNA exclusively in IL-1β-stimulated Müller glial cells

We hypothesize that there is a selective mechanism to regulate galectin-1/*LGALS1* expression in DR eyes. We checked cytokine applications to 3 major cell types closely associated with the pathogenesis of DR: Müller glial cells (MIO-M1), retinal microvascular endothelial cells (HRMEC), and macrophages (THP-1). (**A-C**) *LGALS1* mRNA levels significantly increased solely after IL-1β stimulation to MIO-M1.

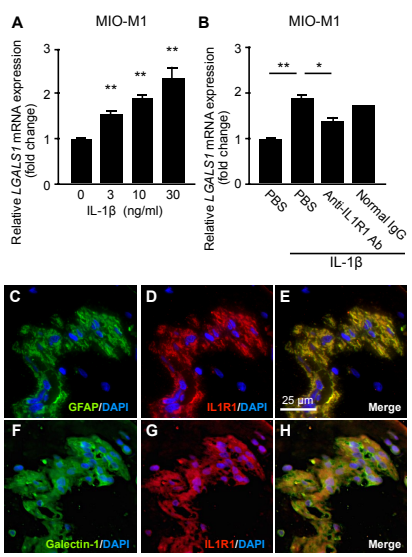

Figure 3. Galectin-1/*LGALS1* expression in Müller glial cells via IL-1β-IL1R1 signaling

(**A**) IL-1β application to MIO-M1 elevated *LGALS1* mRNA levels in a dose-dependent manner. (**B**) The upregulated *LGALS1* mRNA expression was suppressed by pretreatment with anti-IL1R1 neutralizing antibody. (**C-H**) IL1R1 immunoreactivity was strongly positive in the fibrovascular tissue and co-localized with GFAP and galectin-1 signals.

These results suggest that the production of galectin-1 protein via IL1R1 signaling in glial cells migrating into the proliferative tissue.

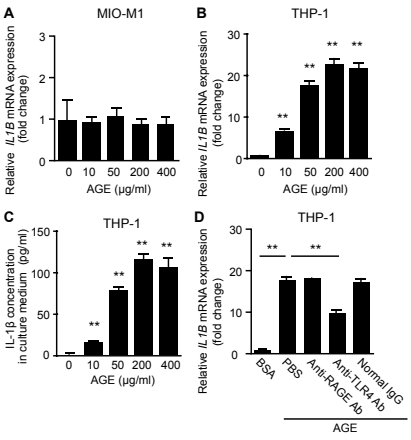

Fig. 4 AGE (advanced glycation end product)-induced expression of IL-1β in macrophages via TLR4 signaling

We hypothesized AGE as a potential inducer for IL-1β because AGE production has been regarded as one of the main contributors for microvascular complications in diabetes. THP-1 clearly demonstrated a dose-dependent responsiveness to AGE in *IL1B* transcripts (**B**) and products in culture media (**C**), but not MIO-M1 (**A**). (**D**) AGE-stimulated IL1B expression was significantly suppressed by pretreatment with anti-TLR4 neutralizing antibody, but not with either anti-RAGE neutralizing antibody or normal IgG.

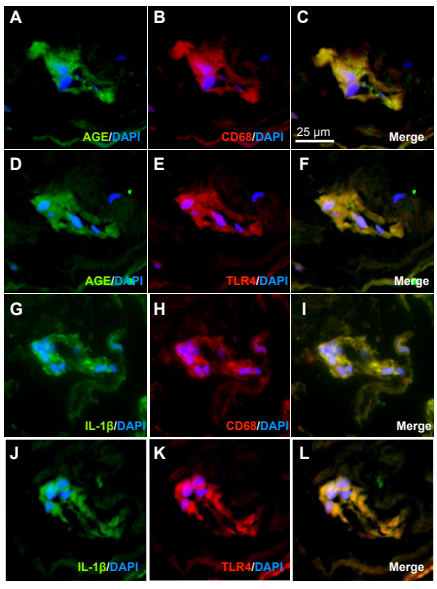

Fig. 5 Tissue co-localisation of AGE, TLR4 and IL-1β in macrophages

(**A-L**) Double-staining experiments demonstrated co-localization of AGE/IL-1β with CD68, and TLR4 in the fibrovascular tissue, suggesting the production of IL-1β protein via AGE/TLR4 signaling in infiltrating macrophages.

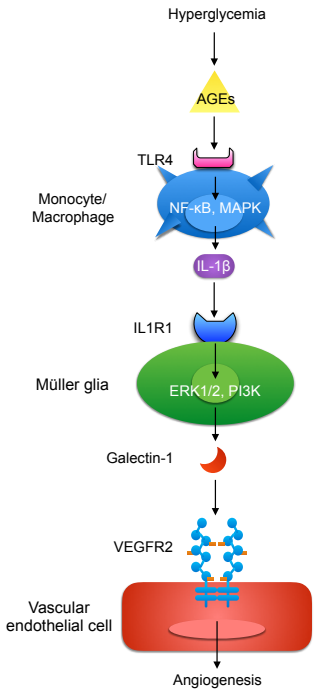

Fig. 6 A schema showing the involvement of AGE-triggered inflammatory cues linking to galectin-1 upregulation in the pathogenesis of DR

AGE accumulation due to prolonged hyperglycemia triggers cellular (macrophage) and molecular (IL-1β) inflammatory cues linking to glial galectin-1 production, explaining the elevation of galectin-1 along with disease activity of DR.

Conclusions: Our results highlight that diabetes-induced AGE accumulation activates IL-1β-related inflammatory cues in macrophages followed by Müller glial cells, linking to galectin-1 upregulation along with the severity and the pathogenesis of DR.

References: (1) Croci DO, Cerliani JP, Dalotto-Moreno T, *et al.*, *Cell* 2014;156:744-758; (2) Kanda A, Noda K, Saito W, Ishida S: *Sci Rep.* 2015;5:1794.
COI: Atsuhiro Kanda, Bayer Yakuhin Ltd (F); Susumu Ishida, Bayer Yakuhin Ltd (F)