**Research Article**

**Nuclear Translocation of GAPDH in Retinal Ganglion Cells is an Early Event in Diabetic Retinopathy**

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**Abstract**

**Purpose:** Recent studies suggest that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mediates the activation of multiple pathways implicated in the pathogenesis of diabetic retinopathy. However, the mechanism by which GAPDH affects retinal cell function is poorly understood. In this study, we analyzed the expression of GAPDH in retinal neuronal cells during the development of diabetes.

**Materials and Methods:** Diabetes was induced in rats by injection of streptozotocin (STZ). At different time points after the induction of diabetes, diabetic and age-matched control rats were killed, eyes were enucleated, and paraffin sections were prepared. The expression of GAPDH was analyzed by immunohistochemistry. To examine the effects of glycemic control, diabetic rats were treated with insulin.

**Results:** In retinas of control rats, GAPDH was mostly localized in the cytoplasm of retinal ganglion cells (RGCs). In contrast, diabetic rats showed nuclear translocation of GAPDH in RGCs. Nuclear GAPDH expression was first detected 4 weeks after the induction of diabetes, and the ratio of GAPDH-positive nuclei to total nuclei increased over time. At 12 weeks, approximately 17% of nuclei were GAPDH-positive, whereas approximately 3% of nuclei in RGCs were GAPDH-positive in control rats. In addition, diabetes-induced nuclear translocation of GAPDH in RGCs was prevented by glycemic control.

**Conclusion:** We demonstrated diabetes induced nuclear translocation of GAPDH in RGCs at an early stage of diabetes in a hyperglycemia-dependent manner. These results help to understand molecular mechanisms of retinal neurodegeneration during diabetic retinopathy development.

**ABBREVIATIONS**

DR: diabetic retinopathy, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, STZ: streptozotocin, GFAP: glial fibrillary acidic protein, RGCs: retinal ganglion cells, ONL: outer nuclear layer, INL: inner nuclear layer

**INTRODUCTION**

Retinopathy is one of the most common complications of diabetes [1]. Diabetic retinopathy (DR) has long been recognized as a vascular disease, and it was believed that the associated visual dysfunction was due to vascular abnormalities [2]. However, recent studies have shown that retinal neurodegeneration is already present before any vascular abnormalities can be detected by ophthalmoscopic examinations [1-3]. Thus, retinal neurodegeneration is an early event in the pathogenesis of DR, and it can predate and participate in the vascular abnormalities that occur in DR patients. Indeed, in diabetic retinas, decreased total retinal thickness [4], as well as a decreased number of retinal ganglion cells (RGCs) in the ganglion cell layer (GCL) has been reported [5]. Thus, visual dysfunctions in diabetes could be explained by degeneration or dysfunction of retinal neural cells. However, the underlying mechanisms of retinal cell death or dysfunction are still largely unknown.

Relatively little is known about the physiological changes of neural and glial cells in diabetic retinas. One feature of retinal damage is reactive gliosis, which is characterized by the up-regulation of glial fibrillary acidic protein (GFAP) in Müller cells [6]. Changes in the expression of GFAP or glial reactivity occur in
many neurodegenerative diseases and are considered a sensitive indicator of retinal damage [7]. Diabetes also increases GFAP expression in human and rat retinas [8-10]. Interestingly, glial activation that occurs in retinas of diabetic patients precedes microvascular abnormalities [11]. However, the function of GFAP expression in Müller cells is not yet clear. In addition, it is not known which of the two primordial pathological features, neurodegeneration or glial activation, occurs first and is consequently the primary event.

Although many glucose-induced retinal metabolic abnormalities are postulated to contribute to DR development, the exact mechanism remains elusive. It was suggested that diabetes-specific microvascular damage is induced through inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described below [12,13]. Hyperglycemia-induced overproduction of mitochondria superoxide inactivates GAPDH in vascular endothelial cells. This inhibition of GAPDH increases the levels of all glycolytic intermediates upstream of 1,3-bisphosphoglycerate. An increased level of the upstream glycolytic metabolite glyceraldehydes-3-phosphate activates the four major pathways of hyperglycemic damage, including increased formation of advanced glycation end products, activation of protein kinase C, and activation of the hexosamine pathway and polyol pathway [12]. Although GAPDH is a glycolytic enzyme and was once considered a simple housekeeping protein, recent studies have shown that it is involved in many cellular processes in addition to glycolysis, including DNA repair, tRNA export, membrane fusion, and cell death [14]. In addition, GAPDH plays a significant role in the development and progression of diabetic retinopathy [15,16]. Nuclear accumulation of GAPDH in rat retinal Müller cell lines undergoing apoptosis due to high glucose concentrations [15]. In addition, retinal GAPDH activity and expression is subnormal with increased ribosylation and nitration in diabetic rats [16]. However, the mechanism by which GAPDH contributes to the pathogenesis of DR is not known.

In this study, we analyzed the expression of GAPDH in retinal neuronal cells during the development of diabetes to investigate the pathology of DR. We also determined the effects of glycemic control on the expression of GAPDH in retinal cells.

MATERIALS AND METHODS

Induction of diabetic rats and insulin treatment

All animal experiments described in this study were handled in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All rats were group-housed in suspended wire-bottomed cages with food and water administered ad libitum, under a 12-h light-dark schedule.

Diabetes was induced in 5-week-old female Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) by a single i.p. injection of streptozotocin (STZ) (Sigma Co., St. Louis, MO, USA) at a dose of 60 mg/kg body weight. Blood glucose concentration was measured from the tail veins using the blood glucose monitoring system NIPRO FreeStyle FREEDOM (Nipro, Osaka, Japan). The development of diabetes was defined as a blood glucose level higher than 400 mg/dl 48 h after STZ injection. Blood glucose and body weight were measured once per week. Confirmed diabetic rats and age-matched control rats were analyzed at 4, 8, 10, 12, and 16 weeks after STZ injection. At least six retinas were analyzed at each time point. Blood glucose values and body weights of diabetic and control rats at the time of sacrifice were assessed (Table 1).

In a further set of experiments, STZ-induced diabetic rats received NPH insulin (Novo Nordisk, Bagsvaerd, Denmark) twice daily (6-10 units total) to maintain their blood glucose levels at below 150 mg/dl and to enable a steady gain in body weight. Blood glucose and body weight were measured twice a week. Diabetic rats, control rats, and insulin-treated diabetic rats were analyzed at 4, 8, and 12 weeks after STZ injection. At least three animals were analyzed at each time point. Blood glucose values and body weights of diabetic and age-control rats at the time of sacrifice were assessed (Table 3).

Immunohistochemistry

At different time points after the induction of diabetes, experimental diabetic and age-matched control rats were killed with carbon dioxide gas. Eyes were enucleated, immediately fixed in methacarn (10% (v/v) acetic acid and 30% (v/v) chloroform in methanol) for 2 h at room temperature and prepared for paraffin sectioning. All sections (3 µm) were subjected to heat-induced antigen retrieval in a pressure cooker for 10 min in 10 mM citrate buffer (pH 6.0). Thereafter, sections were blocked in 10% normal goat serum (Nichirei, Tokyo, Japan) for 30 min at room temperature. Mouse anti-GAPDH antibody (1:4000; Millipore), rabbit anti GAPDH antibody (1:200; Abcam), mouse anti-NeuN antibody (1:50, Santa Cruz Biotechnology), or mouse cy3-labeled anti-glial fibrillary acidic protein antibody (1:1600; Sigma) were incubated for 90 min with secondary antibody Alexa Fluor-568 labeled goat anti-rabbit IgG (1:1000; Invitrogen) or Alexa Fluor-568 labeled goat anti-rabbit IgG (1:1000; Invitrogen) at room temperature. After washing, sections were incubated for 90 min with secondary antibody Alexa Fluor-488 labeled goat anti-mouse IgG (1.500; Millipore), rabbit anti GAPDH antibody (1:200; Abcam), mouse anti-NeuN antibody (1:50, Santa Cruz Biotechnology), or mouse cy3-labeled anti-glial fibrillary acidic protein antibody (1:1600; Sigma) were incubated for 2 h at room temperature. After washing, sections were incubated for 90 min with secondary antibody Alexa Fluor-488 labeled goat anti-mouse IgG (1.500; Invitrogen) or Alexa Fluor-568 labeled goat anti-rabbit IgG (1:1000; Invitrogen) at room temperature. After washing, sections were mounted with ProLong Gold Antifade Reagent (Invitrogen, Carlsbad, CA, USA) and images were taken with a fluorescence microscope (BX51; Olympus).

Analysis of the upregulation of GFAP expression in Müller cells

Diabetic retinas were immunostained with cy3-labeled anti-GFAP antibody and microscopic images were obtained using a fluorescence microscope (BX51; OLYMPUS, Tokyo, Japan). The expression of GFAP was categorized into 4 classes as shown in figure 2 based on the intensity and distribution in Müller cells as follows: [-] GFAP expression was limited to astrocytes, [+] faint GFAP expression was detected in the endfeet of Müller cells, [++] GFAP expression was observed throughout the length of the Müller cells, [+++] intense GFAP expression was detected throughout the length of the Müller cells. To quantify the level of GFAP expression, images were acquired for at least 3 different viewpoints from 4 to 6 diabetic retinas at different durations of diabetes. In each experiment, at least three tissue separated sections were examined and a typical section was selected from every retinal sample. To analyze the expression of GFAP, images were acquired for at least 3 different randomly selected
viewpoints from 4 to 6 diabetic retinas at different durations of diabetes and the distribution and intensity of GFAP expression was determined.

Quantitative analysis of GAPDH-positive nuclei in retinal ganglion cells

Diabetic retinas were immunostained with anti-GAPDH antibody and anti-NeuN antibody and microscopic images were obtained. To quantify the proportion of GAPDH-positive nuclei in retinal ganglion cells, the numbers of NeuN-positive and NeuN/GAPDH double-labeled nuclei in the whole area of the retinal tissue samples were counted. For each duration when diabetes was considered, at least 6 retinas were examined.

Statistical analysis

Statistical analysis was determined differences between control and diabetic rat were evaluated using the Student's t-test. For all analysis, differences at P < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results

Animals: The average weight and blood glucose values for the diabetic and control rats at the each time point are given in Table 1. STZ-diabetic rats had considerably higher weight loss than did age-matched controls at every time point after the induction diabetes. Elevated blood glucose was also observed at the time of sacrifice in STZ-diabetic rats.

Expression of GFAP in diabetic retina: We first examined the expression of GFAP in retinas to evaluate retinal damage. In the retinas of control rats, GFAP expression was limited to astrocytes in the vitreo-retinal border and was not detected in Müller cells (Figure 1). Four weeks after diabetic induction, GFAP expression in diabetic retinas did not differ from that in control retinas (Figure 1b). On the other hand, 8 weeks after diabetic induction, a slight GFAP expression was detected in Müller cells (Figure 1d). After 12 weeks, GFAP expression spread throughout the entire length of the Müller cells and the intensity of GFAP immunoreactivity increased (Figure 1f). Table 2 summarizes the expression of GFAP in diabetic retinas. A slight expression of GFAP in Müller cells was first detected in some retina 6 weeks after diabetic induction. With the progression of diabetes, GFAP expression in the retinas of diabetic rats spread and increased in intensity. Twelve weeks after diabetic induction, remarkable expression of GFAP in Müller cells was observed in every retina.

Diabetes-induced translocation of GAPDH in the nuclei of RGCs: We next analyzed the expression of GAPDH in the retinas of diabetic rats. As shown in Figure 3, GAPDH-positive cells were observed in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) of the control and diabetic retinas. The subcellular localization of GAPDH differed (Figure 3). GAPDH was mostly localized in the cytoplasm in the ONL and GCL, whereas, GAPDH was localized in both cytoplasm and nuclei in INL (Figure 3c, e, g). GAPDH expression of 4-week diabetic retinas did not differ from that of the control retina in the ONL and INL (Figure 3f, h). On the other hand, in the GCL, nuclear localization of GAPDH was observed in the diabetic rat (Figure 3b d, arrow head), whereas no GAPDH positive nuclei was observed in control retina (Figure 3a c). To determine which cells in the GCL showed nuclear GAPDH expression, the retinas from both control and diabetic rats were immunostained with a monoclonal antibody to NeuN, a ganglion cell nuclei marker (Figure 4c, d). In both control and diabetic retina, NeuN antibody specifically reacted with the retinal ganglion cell nuclei, however, other nuclei, such as those of the astrocytes and endothelial cells, showed no immunoreactivity for NeuN (Figure 4c, d). In diabetic

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### Table 1: Blood glucose and body weight of diabetic and age-matched control rats.

<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>n</th>
<th>Blood glucose (mg/dL)</th>
<th>Body weight (g)</th>
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<tr>
<td>4</td>
<td></td>
<td>Normal 3 89 ± 11.5</td>
<td>192 ± 11.4</td>
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<td></td>
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<td>Diabetic 3 442 ± 44.6</td>
<td>120 ± 11.7</td>
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<td>Normal 3 101 ± 9.0</td>
<td>247 ± 7.2</td>
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<tr>
<td></td>
<td></td>
<td>Diabetic 3 452 ± 53.7</td>
<td>177 ± 46.3</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Normal 3 93 ± 2.6</td>
<td>261 ± 18.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 424 ± 31.9</td>
<td>165 ± 34.7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Normal 3 90 ± 4.4</td>
<td>266 ± 13.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 432 ± 33.6</td>
<td>144 ± 14.0</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>Normal 3 90.7 ± 6.4</td>
<td>307 ± 27.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 424 ± 19.1</td>
<td>222 ± 30.6</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Normal 3 107 ± 21.5</td>
<td>312 ± 12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 490 ± 16.7</td>
<td>277 ± 8.5</td>
</tr>
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</table>

The values are mean ± SD.

### Table 2: Expression of GFAP in diabetic retina.

<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>Intensity of GFAP expression</th>
<th># of retina</th>
</tr>
</thead>
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<tr>
<td></td>
<td>-</td>
<td>±</td>
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<tr>
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<td>6</td>
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<td>8</td>
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<tr>
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<td>3</td>
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<tr>
<td>12</td>
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<tr>
<td>16</td>
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</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

The expression intensity was described in the legend of figure 2.

### Table 3: Blood glucose and body weight of diabetic rat after glycemic control.

<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>n</th>
<th>Blood glucose (mg/dL)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4w</td>
<td></td>
<td>Normal 2 102 ± 11.5</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 435 ± 95.2</td>
<td>181 ± 26.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin 3 54.0 ± 34.7</td>
<td>248 ± 14.2</td>
</tr>
<tr>
<td>8w</td>
<td></td>
<td>Normal 3 83.3 ± 3.1</td>
<td>256 ± 27.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 487.3 ± 14.8</td>
<td>222 ± 29.5</td>
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<tr>
<td></td>
<td></td>
<td>Insulin 3 65.3 ± 32.7</td>
<td>203 ± 7.6</td>
</tr>
<tr>
<td>12w</td>
<td></td>
<td>Normal 3 84.3 ± 402</td>
<td>313 ± 18.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic 3 433 ± 3.8</td>
<td>194 ± 44.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin 3 89.7 ± 37.8</td>
<td>303 ± 8.5</td>
</tr>
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</table>

The values are mean ± SD.
Figure 1 Diabetes induces GFAP expression in retinas of diabetic rats. Immunofluorescence staining for GFAP was performed on retinal sections from rats at 4 (b), 8 (d), and 12 (f) weeks after onset of diabetes and age-matched non-diabetic controls (a, c, and e, respectively). DAPI was used to counterstain cell nuclei. Abbreviations: ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; ILM, inner limiting membrane. Scale bar represents 100 μm.

Retinas, all of the nuclear GAPDH-expressing cells were positive for NeuN (Figure 4d, f, h), indicating that some of retinal ganglion cells showed nuclear expression of GAPDH. The expression of nuclear GAPDH in RGCs of diabetic and age-matched control rats was further analyzed for up to 12 weeks after the onset of diabetes (Figure 5 and 6). In diabetic retinas, the mean incidence of nuclear GAPDH was 7.4–17.1% of RGCs (Figure 6). The mean incidence of nuclear GAPDH was less than 2.9% in age-matched control retinas (Figure 6).

Effect of glycemic control on nuclear translocation of GAPDH: We further examined whether nuclear translocation of GAPDH is prevented by glycemic control. For this purpose, diabetic rats were administered insulin. Insulin administration resulted in the maintenance of blood glucose levels of diabetic rats at below 150 mg/dl and prevented weight loss (Table 3). The maintenance of glycemic control in rats decreased retinal GFAP expression in Müller cells (Figure 7A), indicating that retinal damage was prevented. In addition, diabetic-induced GAPDH nuclear localization was also suppressed (Figure 7B). In diabetic rats, 5–12% of RGCs showed nuclear GAPDH expression (Fig. 8). On the other hand, in rats that maintain glycemic control, 1.4–1.8% of RGCs showed nuclear GAPDH expression, and this proportion was not different from that of RGCs in normal retinas (Figure 8).
Figure 2. Classification of the expression level of GFAP in retinas of diabetic rats. The expression of GFAP was categorized into 4 classes based on its intensity and distribution in Müller cells as follows:

- GFAP expression was limited to astrocytes
± faint GFAP expression was detected in the endfeet of Müller cells
+ GFAP expression spread along the entire length of Müller cells
++ intense GFAP expression was detected along the entire length of Müller cells

Figure 3. Expression of GAPDH in diabetic retinas. Retinal sections from rats at 4 weeks after onset of diabetes (b, d, f, and h) and age-matched control rats (a, c, e, and g, respectively) were immunostained with anti-GAPDH monoclonal antibody. The boxed areas of (a) and (b) are shown at higher magnification in (c–h). In the diabetic rats, nuclear localization of GAPDH was observed in the GCL (d, arrowhead). Abbreviations: ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; ILM, inner limiting membrane. Scale bars represent 50 μm.
Figure 4 Diabetes induces nuclear translocation of GAPDH in retinal ganglion cells of the ganglion cell layer. Retinal sections from rats at 4 weeks after onset of diabetes (b, d, f, h) and age-matched control rats (a, c, e, g) were immunostained with anti-NeuN monoclonal antibody (c, d) and anti-GAPDH polyclonal antibody (e, f). Sections were counterstained with DAPI to visualize all nuclei (a, b). Panels g and h indicate merged images. NeuN expression is observed in all GAPDH-positive nuclei. Arrowheads indicate GAPDH-positive nuclei. Scale bar represents 50 μm.

Figure 5 GAPDH-positive nuclei were detected in retinal ganglion cells of diabetic rats. Sections were obtained from age-matched control rats (a,c,e,g,i) and rats at 4 weeks (b), 6 weeks (d), 8 weeks (f), 10 weeks (h), and 12 weeks (j) after onset of diabetes. Retinal sections were immunostained with anti-GAPDH polyclonal antibody. Arrowheads indicate GAPDH-positive nuclei. Scale bar represents 50 μm.
Figure 6 Quantitative analysis of the percentage of GAPDH-positive nuclei in retinal ganglion cells of diabetic rats.
A. Control and diabetic retinas were immunostained with anti-GAPDH antibody and anti-NeuN antibody. NeuN-positive/GAPDH-negative (a) and NeuN-positive/GAPDH-negative (b) nuclei were observed.
B. The numbers of NeuN-positive or NeuN/GAPDH double-labeled nuclei in the whole area of retinal tissue samples were counted, and the percentage of NeuN/GAPDH double-labeled nuclei at different time points after the initiation of diabetes (n = 4–6 retinas per group) was obtained.
The horizontal line represents the mean. Black triangle: diabetes; White Square: age-matched control. The horizontal line represents the mean. * and ** represent a statistically significant difference at p<0.05 and p<0.01, respectively.

Discussion

In this study, we demonstrated nuclear translocation of GAPDH in retinal ganglion cells only 4 weeks after diabetes induction. This nuclear translocation was prevented by glycemic control. Thus, the nuclear translocation of GAPDH in RGCs was a hyperglycemia-induced event. To our knowledge, this is the first evidence of a histological change in RGCs during the early stage of diabetes.

The expression of GFAP in Müller cells, which is a cellular marker for retinal damage [17], increased in diabetic retinas. Previous studies demonstrated GFAP expression in rats suffering from diabetes for more than 3 months [9,10,17,18]. Thus, retinal damage detected by the expression of GFAP in diabetic rat starts about 3–4 months after the induction of diabetes. We also detected GFAP expression spanning the entire length of Müller cells in all retinas after 10 weeks of diabetes. However, functional impairment of the diabetic retinas was observed in the early period of diabetes. Electoretinographic abnormalities are present 2 weeks after inducing diabetes [17]. Thus, early functional impairment will precede followed by GFAP expression. Our results indicated that nuclear GAPDH in RGCs was present as early as 4 weeks after the induction of diabetes. This suggests that the nuclear translocation of GAPDH in RGCs reflects the pathological process of early diabetes and affects retinal functions. However, the precise function of nuclear GAPDH in RGCs is not clear.

A recent study has demonstrated that cytoplasmic GAPDH
Figure 7 A: The effect of glycemic control on GFAP expression in retinas. Immunofluorescence staining for GFAP was performed on retinal sections from rats at 12 weeks after onset of diabetes (b), rats at 12 weeks after onset of diabetes with maintained glycemic control (c), and age-matched non-diabetic controls (a). Scale bar represents 100 μm. B: Expression of GAPDH in the retinal ganglion cell layer from diabetic rats maintaining glycemic control. Retinal sections from rats at 4 weeks of onset of diabetes (b, e), diabetic rats at 4 weeks of onset with maintained glycemic control (c, f), and age-matched control rats (a, d) were immunostained with anti-GAPDH monoclonal antibody. Scale bar represents 50 μm.

Figure 8 The effect of glycemic control on nuclear translocation of GAPDH in retinal ganglion cells. The numbers of DAPI stained and GAPDH-labeled nuclei in the whole area of retinal tissue samples were counted, and the percentage of GAPDH-labeled nuclei at different durations of diabetes (n = 4–6 retinas per group) was obtained. Black triangle: diabetes; white square: age-matched control; gray circle: glycemic control. The horizontal line represents the mean. ** represents a statistically significant difference at p<0.01.
is translocated to the nucleus upon exposure to stressors and participates in cell death or dysfunction [14]. In addition, S-nitrosylation by NO at active site Cys-150 allows GAPDH to bind to Siah (an E3 ubiquitin ligase) and leads to nuclear translocation of the GAPDH-Siah complex, which results in cellular dysfunction and death [19]. Several histological studies have demonstrated that RGCs seem to be lost in diabetic patients [11,20,28]. In addition, RGC loss or damage was also observed in experimentally induced diabetic rats [5,17]. Barber and colleagues [28] reported that the number of apoptotic nuclei of RGCs is elevated after 1 month of STZ-induced diabetes and the thickness of the IPL decreases after 7.5 months of STZ-diabetes. These results raise the possibility that nuclear GAPDH in RGCs may be involved in cell death. However, the relationship between nuclear translocation and RGC cell death is unclear. Thus, further investigation is needed.

Although a progressive loss of cells observed in DR, apoptotic cell death of retinal cells may have a small effect for loss of visual function, especially in the early stages of disease progression, because the number of cells dying in diabetic retinas is very small [1]. Indeed, nuclear GAPDH possesses various functions unrelated to cell death [14]. GAPDH plays a critical role in DNA repair, both directly [21,22] and indirectly [23]. Nuclear GAPDH interacts with DNA as a coactivator of Oct-1 to stimulate the expression of Histone 2B [4]. Furthermore, nuclear GAPDH also plays a role in maintaining and protecting telomeric DNA from rapid degradation [24]. Because of its prominent expression, GAPDH can function as a sensor of intra- and extra-cellular stresses [14]. Many studies have indicated that a pool of GAPDH translocates to the nucleus under a variety of stressors, especially oxidative stress [25]. In addition, cellular dysfunction or cell death in DR is caused by oxidative stress induced by hyperglycemia [1,26]. Thus, nuclear GAPDH may be involved in several cell protective functions at the early stage of diabetes.

CONCLUSION

In conclusion, we showed the translocation of GAPDH to the nucleus in RGCs of rats with diabetes. This nuclear accumulation of GAPDH in RGCs begins at an early stage of diabetes. A recent report has identified small-molecule compounds with demonstrated anti-apoptotic activity that selectively interacts with GAPDH [27]. Moreover, deprenyl, a drug for patients with Parkinson’s disease, and derivatives of deprenyl block nuclear translocation of GAPDH [14,15,25]. Thus, understanding the molecular mechanisms of GAPDH nuclear translocation will facilitate the development of a new therapeutic strategy to prevent DR.

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