Diabetic Complications Consortium

Application Title: Role of crystallins in the neurodegenerative and neuroinflammatory components of human DR

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1. Project Accomplishments:

Our primary goal was to test the general hypothesis that α-crystallins are upregulated in retinal Müller cells during diabetes in response to retinal inflammation and neurodegeneration. The specific goal of this proposal was to determine the relationship of crystallin protein expression with neurodegeneration and neuroinflammation in human diabetic retinopathy. Using our biorepository of human ocular samples from donors with and without diabetes or retinopathy, we showed that: 1) αA- and αB-crystallins are upregulated in a regional manner in the retina of diabetic patients, and that as a function of the disease progression; 2) diabetes leads to a specific reduction of the retinal thickness in absence of proliferative retinopathy or diabetic edema, in the inferior quadrant especially, and before any clinical signs of retinopathy; 3) diabetes leads to a loss of Müller glia, the main glial cells of the retina; 4) specific loss of growth factor signaling and increased inflammation markers are associated with neurodegeneration in diabetic donors.

Specific Aims:

This project of identifying the relationship between neurodegeneration and neuroinflammation and α-crystallins expression was tested using 2 aims on which substantial progress were made during the funding period.

For Aim 1: Determine the relationship of α-crystallin expression with loss of retinal cells in human retina. We had hypothesized that diabetes-induced retinal neurodegeneration is associated with a cellular specific increased expression of numerous crystallin proteins in human ocular tissues. In order to test this hypothesis we performed immunoblot analysis of the expression of several crystallin proteins including αA-, αB-, βB2- and βA3/A1-crystallins in central versus peripheral retina. This analysis clearly demonstrated that both the region and the stage of the disease affected the expression of crystallin proteins with a significant induction of αA-crystallin in the central retina, while αB-crystallin is specifically found to be induced in the peripheral retina of donors with diabetes (Figure 1). In both cases, the effect is slightly enhanced in donors with diabetic retinopathy but not significantly supporting that this response is an adaptive protective response, potentially failing over time. In order to test this part of the hypothesis, we used mass spectrometry to quantify specific post-translational modification affecting αA-crystallin. Using multiple reaction monitoring, we quantified the phosphorylation level of αA-crystallin in retinal lysate from non-diabetic and diabetic donors with diabetic retinopathy and uncovered that this phosphorylation was dramatically reduced by over 80% in diabetic donors with retinopathy (Figure 2) supporting that this phosphorylation could be an important regulator of the protective function of αA-crystallin, which is currently under further investigation using cell culture and animal models.

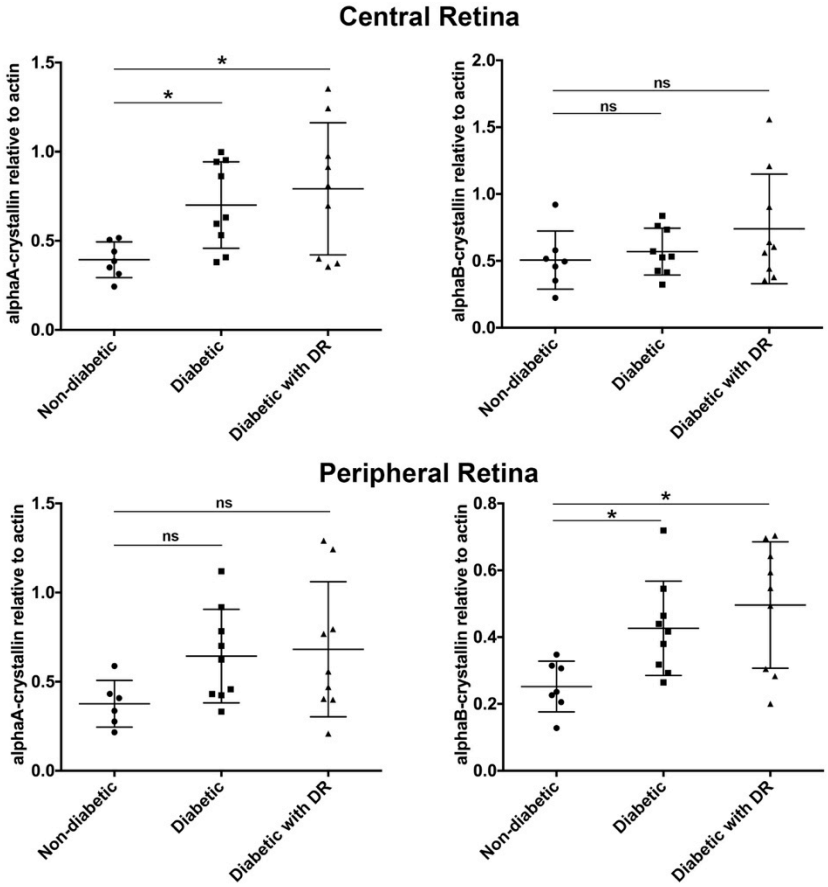

Figure 1: Diabetes increases retinal crystallins expression in a regional manner. Western-blot analysis was performed on central and peripheral retina tissues from non-diabetic, diabetic without retinopathy and diabetic patient with mild to moderate DR. The results demonstrate a specific induction of αA- and αB-crystallin in the central and peripheral retina respectively.

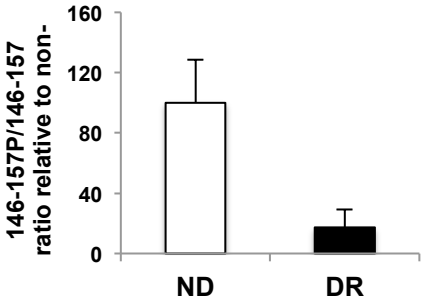

Figure 2: Diabetes specifically reduces phosphorylation on residue 148 of αA-crystallin. Absolute quantification of the phosphorylation of αA-crystallin on residue 148 was performed by multiple reaction monitoring and showed >80% reduction in samples from human donors with retinopathy.

One of the goals of this study was to assess how these changes in crystallins expression were associated with neurodegeneration and neuroinflammation. In order to do so, we performed immunohistochemistry analysis of the contralateral eye of the same donors used for biochemical analysis and showed that those changes in expression were associated with a specific thinning of the retina of diabetic donors, especially localized in the inner retina and particularly affecting the superior quadrant (figure 3).

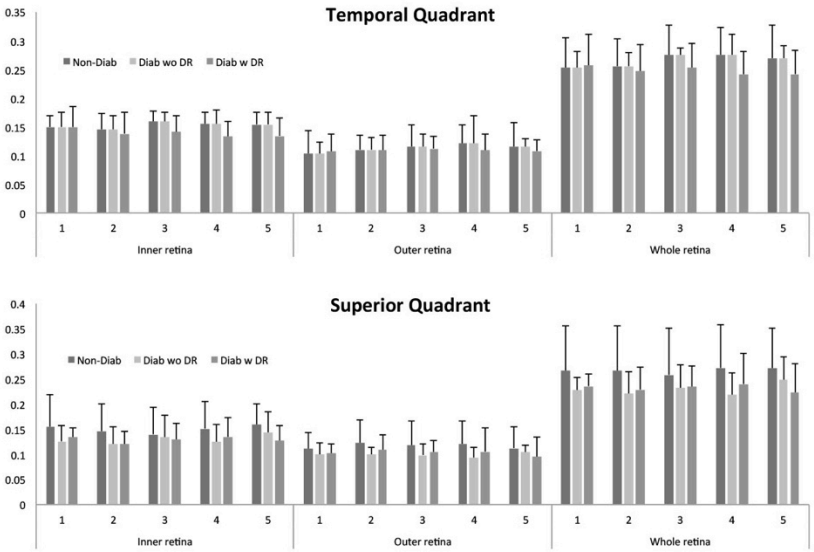

Figure 3: Diabetes is specifically associated with a thinning of the retina, independent of clinical signs of diabetic retinopathy, and more specifically in the superior quadrant of the retina. Retinal thickness was measured on OCT (optical coherence tomography) images obtained post-mortem and showed a thinning of the inner retina in patients with diabetes (with or withour retinopathy), particularly of the peripheral retina in the superior quadrant.

Further studies are currently ongoing to expand this analysis and perform more cell-specific analysis.

For Aim 2: Determine if α-crystallin expression in human retina is associated with neuro-inflammation. Our main goal was to test the hypothesis that α-crystallins upregulation is directly involved in the regulation of the neuroinflammatory response associated with diabetic retinopathy. In order to do so, we embarked to assess how micro and macro-glial cells are affected by diabetes and diabetic retinopathy. We first assessed the impact of diabetes on the survival of Müller glial cells, the main macroglial cells in the retina. Using immunostaining for a specific cellular marker of MGCs (glutamine synthetase), we counted the number of cells and our preliminary data strongly suggest a reduction in the number of MGCs in the retina as a function of the progression of the disease and the onset of DR (Figure 4).

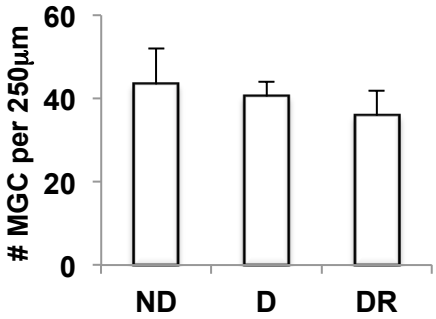

Figure 4: Diabetes is associated with a reduction in the number of Müller glial cells, especially in patients with diabetic retinopathy. Retinal cross-sections of the central retina of donors were immunostained with glutamine synthetase, a Müller glial cell specific marker. Cell bodies were identified by colocalization with counterstaining with Dapi for cell nuclei. Quantification in retinal sections from 4 non-diabetic, 2 diabetic without retinopathy and 4 diabetci with retinopathy strongly support the trend of a progressive reduction in the number of MGCs in the central retina of diabetic donors, especially with retinopathy.

We further analyzed the progression of the glial and inflammatory response to diabetes and its relationship to onset and development of retinopathy. In order to do so, we analyzed the glial response using GFAP, a marker of glial activation known to be induced in activated Müller glial cells, and Iba1, a marker of inflammatory cells, primarily staining resident microglial cells the same donors with retinopathy in which we identified increased levels of α-crystallins and decreased phosphorylation, we saw regions of large increased glial activation in conjunction with increased microglial cell numbers (Figure 5). In comparison, donors without retinopathy and without diabetes altogether showed low levels of glial activation and lower number of microglial cells. While these data remain to be confirmed in a larger number of sample, this is consistent with the biochemistry and mass spectrometry findings supporting our hypothesis that α-crystallins are directly involved in the glial activation and regulation of inflammation observed in diabetic patients with retinopathy.

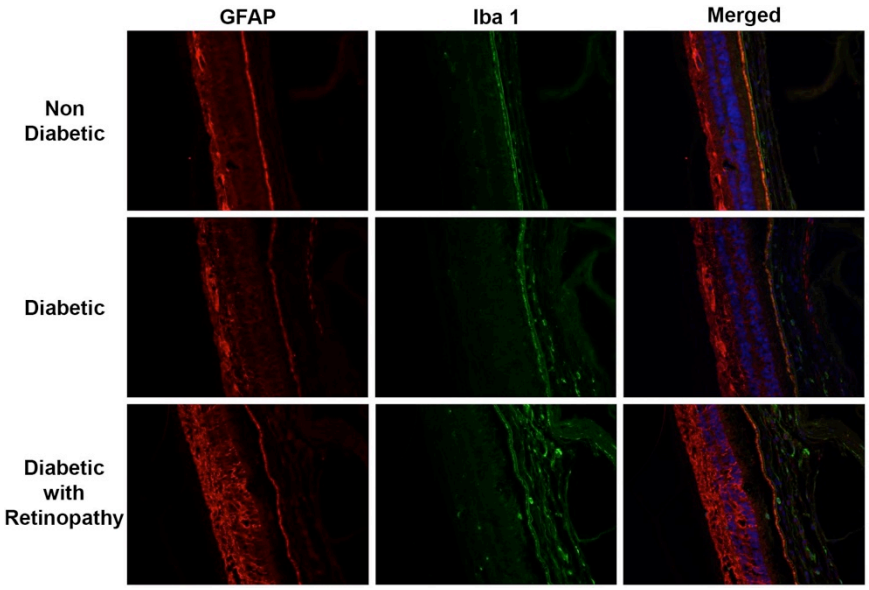

Figure 5: Donors with retinopathy, but not diabetes alone, exhibit increased glial activation and increased number of inflammatory cells. Retinal cross-sections of the peripheral retina of donors were immunostained with GFAP, a marker of activated Müller glial cells and Iba1, a marker of inflammatory cells (resident and recruited).

2. Publications:

N/A