OBSERVATIONS

New Pathogenic Candidates for Diabetic Macular Edema Detected By Proteomic Analysis

Diabetic macular edema (DME) is the main cause of visual impairment in diabetic patients. The aim of the present study was to explore the differential proteomic pattern of the vitreous fluid from DME patients by means of proteomic analysis. For this purpose, we used fluorescence-based difference gel electrophoresis (DIGE). This technique provides accurate quantitative comparisons, and we further optimized it in order to avoid potential confounding factors (1).

Samples of vitreous from eight type 2 diabetic patients (four with DME without proliferative diabetic retinopathy [PDR] and four with PDR without DME), and eight from nondiabetic subjects with idiopathic macular hole (control group) were selected from our vitreous bank for proteomic analysis.

The protocol for sample collection was approved by the Vall d'Hebron hospital ethics committee, and informed consent was obtained from the patients. The study was conducted in accordance with the principles of the Declaration of Helsinki. Sample preparations, along with the details of DIGE proteomic analysis, have been described elsewhere (1).

Approximately 1,300 protein spots were detected. By selecting an abundance ratio of 1.5-fold as the threshold for the study, at least 81 spots were differentially produced ($P < 0.05$) between any two of the three groups evaluated. Following matrix-assisted laser desorption ionization/mass spectrometry time-of-flight/time-of-flight analysis, we were able to identify 25 intravitreal proteins (data available from the author upon request). Four of these proteins were specifically associated with DME. This means that there were significant differences between DME versus control subjects and DME versus PDR. These proteins were hemopexin, beta crystalline S, clusterin, and transthyretin. Hemopexin abundance was significantly increased in the vitreous fluid of patients with DME in comparison with PDR and control subjects. By contrast, beta crystalline S, clusterin, and transthyretin were significantly lower in DME patients than in PDR patients and control subjects. In view of the current information, hemopexin and clusterin seem to be more directly related to the development of DME.

Hemopexin is the best-characterized permeability factor in steroid-sensitive nephrotic syndrome (2). T-cell–associated cytokines like tumor necrosis factor-α are able to enhance hemopexin production in mesangial cells in vitro, and this effect is prevented by corticosteroids (3). It should be noted that proinflammatory cytokines have been involved in the development of DME and, therefore, hemopexin might be a mediator of the disruption of the blood-retinal barrier.

Clusterin is associated with protection from apoptotic retinal cell death (4). Recently, Kim et al. (5) demonstrated that clusterin effectively inhibited vascular endothelial growth factor–induced hyperpermeability in human retinal microvascular endothelial cells and in retinal vessels from streptozotocin-induced diabetic mice. Since clusterin plays an essential role in restoring tight junctions and limiting the inflammatory response after injury (two capital features in the pathogenesis of DME), it seems reasonable to propose clusterin deficit as a contributor to DME development.

In summary, we have shown that two-dimensional DIGE analysis facilitates the identification of new potential candidates involved in the development of DME. Further studies addressed to the evaluation of the precise role of these candidates in the pathogenesis of DME and their potential as therapeutic targets would appear to be warranted.

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References

1. García-Ramírez M, Canals F, Hernández C, Colomé N, Ferrer C, Carrasco E, García-Arumí J, Simó R. Proteomic analysis of human vitreous fluid by fluorescence-based difference gel electrophoresis (DIGE): a new strategy for identifying potential candidates in the pathogenesis of proliferative diabetic retinopathy. Diabetologia 2007;50:1294–1303
2. Cheung PK, Klok PA, Baller JF, Bakker WW. Induction of experimental proteinuria in vivo following infusion of human plasma hemopexin. Kidney Int 2000;57:1512–1520
3. Kapojos JJ, van den Berg A, van Goor H, te Loo MW, Poelstra K, Borghuis T, Bakker WW. Production of hemopexin by TNF-alpha stimulated human mesangial cells. Kidney Int 2003;63:1681–1686
4. Kim JH, Kim JH, Yu YS, Min BH, Kim KW. The role of clusterin in retinal development and free radical damage. Br J Ophthalmol 2007;91:1541–1546
5. Kim JH, Kim JH, Yu YS, Min BH, Kim KW. Protective effect of clusterin on blood-retinal barrier breakdown in diabetic retinopathy. Invest Ophthalmol Vis Sci 2010;51:1659–1665