



RBP3: a possible prognostic marker and therapeutic target in diabetic retinopathy

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Is RBP3 just another common piece in the puzzle of diabetic retinopathy, or could it be a key card for its understanding and maybe even treatment?

Chronic progressive pathologies, such as diabetes, are hard to manage and treat, because too little is known of their etiology (partly genetic, partly environmental); early diagnosis can be hard to achieve, and reliable specific prognostic markers are missing, with the exception of a generic index of the glycemic control such as glycosylated hemoglobin (HbA_{1c}). In fact, blood sugar control and insulin therapy is mostly all that is available nowadays to manage diabetes and decrease the risk of one of its most devastating consequences: proliferative diabetic retinopathy. PDR is among the main causes of blindness in the western world, and sure enough a deeper understanding of its molecular pathology will be useful to increase our ability to follow and maybe treat this disease. Several decades of research in this field have generated a tree of risk factors and some biomarkers that are variably associated with the disease and its progression. Arterial hypertension (1), oxydative stress (2), longer duration of diabetes mellitus, type 1 *vs.* 2 of diabetes mellitus, bad metabolic control (i.e., high HbA_{1c} level), and shorter axial length (hyperopia) are the accepted risk factors (3). The inflammatory events that follow the metabolic dysregulation may lead to the accumulation of inflammatory cytokines in the vitreous body (4), triggering autophagy and apoptosis in retinal pigment epithelial cells (RPE), essential to photoreceptor survival (5). Metabolic

profiling of blood or vitreous body of PDR patients have indicated several biomarkers associated with disease progression (6,7). The presence of specific microRNAs has been linked to PDR evolution (8,9). However, none of the above elements can be reliably used as individual prognostic marker, even less as a specific therapeutic target. More recently, angiopoietin-like 3 (ANGPTL3) has been described as independently and strongly associated with DR progression in all stages. Therefore, blockade of ANGPTL3 signal in retina might postpone the onset and development of DR in type 2 diabetic patients (10). However, no proof of concept exists as yet to this inference. With a different strategy, our research group has exploited the anti-angiogenic and antinflammatory properties of a synthetic peptide (UPARANT: Urokinase Plasminogen Activator Receptor ANTagonist) designed to interfere with the downstream signaling of the formyl peptide receptor (FPR) and associated proteins, to partially rescue the retinal degeneration in animal model systems of type I (11) and type II (12) diabetes, thus showing the relevance of the UPAR/FPR system in the vascular dysregulation occurring during diabetes progression and DR (13).

Despite intensive research into most aspects of metabolic diseases including diabetes mellitus and its complications, much work remains to be done to identify accessible predictive biomarkers that can guide the pharmacological treatment. In this respect, most research groups are directing their studies toward the identification of potential

circulating biomarkers with precise prognostic value (14). Several molecular biomarkers show promise as screening markers to detect early diabetic retinopathy or even to detect patients at increased risk of diabetic retinopathy at the time of diagnosis of diabetes. Among molecular tools, scientists have recently shifted their research focus towards non coding RNA molecules with the aim of developing novel specific biomarkers for early detection of metabolic disorders and their complications. Little is known on the functional properties of non coding RNAs although there are some indications that they may interact with RNA-binding proteins (RBPs) to contribute to the pathogenesis of diverse metabolic processes including their ophthalmological complications (15). In diabetic retinopathy, for instance, there are some findings indicating that non coding RNAs participate to the regulation of endothelial cell function through major effects on retinal levels of VEGF (16). Although much work remains to be done to clarify the role of non coding RNAs as sensors and biomarkers for metabolic disorders, the possibility to correlate their plasmatic levels with the severity of the disease remains very exciting.

In the context of predictive biomarkers a recent paper of Yokomizo *et al.* (17) based on previous work done by other groups (18-20), emphasized the importance of the retinol binding protein RBP3 (exclusively produced by retina photoreceptors) as differentially present in the retina and the vitreous humor of mostly type I diabetic patients affected by different degrees of DR. High amounts of RBP3 in the retina and the vitreous correlated with no or low grade DR, low amounts with the presence of PDR. Most strikingly, artificially induced overexpression of RBP3 *in vivo* or *in vitro* attenuated DR and its related events. A mechanism by which RBP3 appears to exert its effects is RBP3 antagonistic binding to GLUT1, a major glucose transporter through the blood-retinal barrier. GLUT1 has been identified as a promising target for DR since the inhibition of its expression decreases the retinal glucose concentration in diabetic mice and ameliorates the pathological signs of diabetic retinopathy (21). The overexpression of RBP3 would lead to the inhibition of glucose uptake in retinal cells with subsequent decreased expression of VEGF and inflammatory cytokines (17). Therefore, RBP3 appears to be not only another biomarker associated with DR, but a key player in its development and progression, and a possible element to exploit in therapy.

Too good to be true? In fact, in order to bring this discovery from the bench to the bedside, as translational

research is supposed to do, there are still some relevant matters to be better elucidated.

RBP3 is found in the retina and the vitreous; only a tiny amount of unknown origin is present in the blood (though detectable by a high-sensitivity ELISA). In order to be used as a prognostic biomarker in living individuals, RBP3 must be detected in accessible peripheral fluids. Therefore, it is important to know what is its distribution in the population: whether the high-sensitivity ELISA will allow to identify and discriminate groups with different serum concentrations of RBP3, and how stable in time is such asset: is it age and/or life-style dependent? And this leads to another key question: are the different amounts of RBP3 found in the retina and the vitreous a cause or an effect of DR progression? If individuals were endowed with different expression abilities of RBP3, it might be plausible that its molecular functions as defined by the study of Yokomizo *et al.* may protect the retina from degenerative events, conferring to their possessors a higher resistance to DR progression. On the other hand, if RBP3 were found to be expressed at similar levels in all individuals, so that the initial degree of protection is comparable, then it could be that its susceptibility to degradation is different. In this case, RBP3 would be less degraded (and more protective) in the slow-progressors, and more degraded (and less protective) in fast progressors, so that patients with PDR have less residual RBP3 and more degradation products, as data in this paper might suggest. This hypothetical different resistance of RBP3 to degradation might depend on allelic variations of the protein, or on upstream events impinging on RBP3 stability.

Another key point is whether in diabetic patients different serum concentrations of RBP3 may also represent a risk factor for progression. Indeed, it could already be possible from the data obtained in this published study to see whether there is a hint of correlation between RBP3 concentration in serum and in the eye, and therefore with the likelihood of PDR. In such case, a prospective study would be feasible, to address the question as to whether progression of DR in an early diabetic population correlates to RBP3 expression. Alternatively, expression genetics of RBP3 and its allelic variants (if any) could be addressed, with the same goal of validating RBP3 as a prognostic marker. However, even though it could not be validated as such, the observation remains that RBP3 overexpression is protective against DR, and therefore it could be a tool to be exploited to delay or soften the adverse events of long-term diabetes and hyperglycemia. To this purpose, the regulation

of RBP3 expression could be addressed, in order to find strategies—if possible—to enhance its expression by means of natural or artificial drugs.

In the past, another retinol binding protein (RBP4) has been linked to diabetes and retinal degeneration, in an opposite way to RBP3 (22,23). RBP4 is an adipocyte-derived ‘signal’ that may contribute to the pathogenesis of type 2 diabetes (24). In fact, serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes. RBP4 circulates in the plasma bound to its carrier protein transthyretin (TTR), which prevents its clearance through the kidneys (25). Its expression appears to be linked to that of the glucose transporter GLUT4. Transmembrane transport of glucose by GLUT4 is the rate-limiting factor for glucose transport in adipose tissue and skeletal muscle and downregulation of GLUT4 expression is characteristic of insulin-resistant states, including obesity, type 2 diabetes and the metabolic syndrome. RBP4 expression increases when GLUT4 expression goes down (26,27). Therefore, it appears that the axes RBP4/GLUT4 and RBP3/GLUT1 work in different ways with opposing results: lowering of RBP4 could be a strategy for treating type 2 diabetes (and indeed, as a proof of concept, a non-retinoid antagonist of RBP4 has been shown to rescue the phenotype in a model of Stargardt disease (28), whereas it is an upregulation of the axis RBP3/GLUT1 which could be beneficial to type I (and maybe also type II) diabetes. The relationship between RBP3 and TTR, if any, remains to be elucidated.

In conclusion, RBP3 appears to have the characteristics of a turning point in the understanding of DR and its progression; whether it will turn out to be a critical milestone, or just another cobblestone remains to be shown.

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Footnote

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