The Role of Müller Cell Glucocorticoid Signaling in Diabetic Retinopathy

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Abstract

Diabetic Retinopathy (DR) is a sight-threatening complication associated with the highly prevalent diabetes disorder. Both the microvascular damage and neurodegeneration detected in the retina caused by chronic hyperglycemia have brought special attention to Müller cells, the major macroglia of the retina that are responsible for retinal homeostasis. Given the role of glucocorticoid signaling in anti-inflammatory responses and the almost exclusive expression of glucocorticoid receptors (GR) in retinal Müller cells, administration of corticosteroid agonists as a potential treatment option has been widely studied. Although these approaches have been moderately efficacious in treating or de-escalating DR pathomechanisms, there are various side effects and gaps of knowledge with regards to introducing exogenous glucocorticoids to the diseased retina. In this paper we provide a review of the literature concerning the available evidence for the role of Müller cell glucocorticoid signaling in DR and we discuss previously investigated approaches in modulating this system as possible treatment options. Furthermore, we propose a novel alternative to the available choices of treatment by using gene therapy as a tool to regulate the expression of GR in retinal Müller cells. Upregulating GR expression allows for induced glucocorticoid signaling with more enduring effects compared to injection of agonists. Hence, repetitive injections would no longer be required. Lastly, side effects of glucocorticoid therapy such as glucocorticoid resistance of GR following chronic exposure to excess ligands or agonists can be avoided.

Key words: Diabetic retinopathy; glucocorticoid signaling; Müller cells, gene therapy

I. Diabetic Retinopathy

According to the World Health Organization, 8.5% of adults aged 18 and older suffer from diabetes and its complications worldwide. Moreover, prevalence of diabetes has been rising at a faster rate in middle- and low-income countries [1]. Approximately a third of people with diabetes are diagnosed with diabetic retinopathy (DR) and a tenth develop vision threatening effects [2], making DR the leading cause of blindness among working-age adults [3]. This prevalence is higher amongst type 1 diabetic patients with 77.3%, compared to 25.2% in type 2 diabetes [3]. Given the pervasiveness and the extensive burden of sight threatening diseases, research in DR and its relevant therapeutic approaches has been a crucial area of interest for scientists.

Retinal damage is a major complication associated with diabetes, though underlying mechanisms are still not completely understood. This is mainly because the tissue response is complex and investigation thereof is hampered by the lack of animal models that recapitulate all disease stages (reviewed in [4,5]). Generally, the insufficiency in insulin production in type 1 diabetes or diminished insulin sensitivity in type 2 diabetes lead to increased blood glucose levels [6]. Hyperglycemia present in diabetic individuals (determined on basis of HbA_{1c} levels) is considered to be one of the main sources of damage to retinal blood vessels which involves pericyte drop-out, formation of acellular capillaries and subsequent tissue responses to local hypoxia that beyond others include neovascularization [7]. Although recent findings question HbA_{1c} as the main and only driver of DR onset or progression [8], most hypotheses on how DR develops ground on hyperglycemia as a starting point. In an endothelial cell-centric view, it is suggested that metabolic imbalances lead to mitochondrial superoxide overproduction and subsequent stimulation of several parallel and potentially independent pathways such as formation of advanced glycation end products (AGEs), upregulation of the AGE receptor RAGE [9] and consequently an over-activation of the hexosamine pathway. As a common endpoint, all these pathways result in enhanced formation of reactive metabolites such as reactive carbonyl, oxygen (ROS) and nitrogen (RNS) species leading to cell death, while altering the tissue response upon hypoxia and contributing to the formation of “hyperglycemic memory” due to long-lasting epigenetic changes [5, 10, 11, 12].

Recent studies propose an additional concept to explain how diabetes might impact tissue integrity, independent from the effects of hyperglycemia. They suggest that an imbalance in the activity of the glyoxalase 1 (GLO1) and 2 system forms and detoxifies the AGE precursor methylglyoxal which is cytotoxic and can damage proteins and DNA [5, 13, 14, 15]. Interestingly, involvement of Müller cells, the major macroglia of the retina, and microglia, resident tissue macrophages, have been implicated in DR-associated pathomechanisms. They express specific transient receptor potential canonical (TRCP) channels that seem to repress GLO1 expression. *Trpc1/4/5/6* knockouts proved to be protective in a mouse DR model and led to higher GLO1 expression with a concomitant lowering of plasma methylglyocal levels [16]. Accordingly, DR pathology does not only originate from functional changes of pericytes and endothelial cells, but may also be due to dysfunction of the glial component of the neurovascular unit.

Finally, unlike former belief, vision loss in DR is no longer considered to be solely a microvascular complication and is also known as a neurodegenerative disease [17]. Hence, the irreversible early-stage neuronal dysfunction and apoptosis prior to the development of vascular pathologies in DR require neuro-protective strategies as soon as diabetes is diagnosed. Retinal insulin receptors not only mediate glucose transport into the cell, but are also involved in stimulating neuronal development, growth and survival [18]. Thus, both reduced insulin receptor signaling and systemic hyperglycemia lead to apoptosis in retinal neurons, particularly ganglion cells, at early stages of diabetes [19, 20].

In proliferative DR, which is the more advanced stage of the disease stemming from chronic hyperglycemia, microvascular leakage of retinal capillaries and neovascularization are recognized as two of the main sources of vision loss [21]. Primarily, the leaking fluid from the damaged blood vessels accumulates in the macula, leading to macular edema. Given the significance of the macula for clear central vision, fluid buildup and swelling in this area distorts vision substantially [22]. Vascular leakage also results in overproduction of ROS, escalating oxidative stress and hypoxia in the retina [23]. Under hypoxic conditions, the hypoxia-inducible factor (HIF) that is normally oxidized by hydroxylase enzymes, is no longer degraded and begins to accumulate. Accumulation of HIF consequently induces the activation of multiple downstream target

genes including vascular endothelial growth factor (VEGF) [24]. Secondly, in an effort to replace the damaged vessels of the retina, the released VEGF will induce the formation of new blood vessels, a process referred to as neovascularization [25]. The atypical and tenuous newly formed vessels themselves cause blood leakage on the surface of retina and into the vitreous which further impairs vision.

Given the complexity of DR pathomechanisms, there is currently no animal model in which all neuronal and vascular complications associated with each stage of this disease are recapitulated as they are found in human patients, especially because typical animal models lack a macula and thus mechanisms of diabetic maculopathy cannot be investigated [4, 5]. Additionally, none of the animal models develop signs of proliferative retinopathy [26]. Nonetheless, several induced (drug or diet-based) and genetic animal models have been developed to represent different stages of the disease, allowing for a better understanding of various aspects of DR etiology, progression, pathology and treatment development. Hyperglycemia and its impact on retinal vasculature and morphological integrity have been mainly modeled in rodents, dogs and non-human primates as they exhibit comparable effects to humans [4]. Although retinal vascular leakage and angiopathies are comparably well represented, neovascularization was not observed in any of those models. To study neovascularization, either models of oxygen-induced retinopathy in the postnatal retina or laser-damage models have been implemented in mice, rats, zebrafish and dogs [4]. Consequently, potential novel treatments must target multiple aspects of DR pathology and should ideally be verified for their efficiency in different preclinical DR models before being considered for validation in clinical trials.

II. Müller Cells

Müller cells, as mentioned above, are the major glial cell type in the retina with a broad variety of significant functions essential for retinal homeostasis that seem to get partially distorted in diabetic individuals. These functions include uptake and recycling of neurotransmitters, control of metabolism and supply of nutrients for the retinal neurons, blood flow regulation and blood retinal barrier (BRB) maintenance [27, 28]. The distinctly

long structure of these cells allows them to span the entire width of the retina and interact with retinal neurons and blood vessels. Hence, proper functioning of Müller cells is crucial in maintaining a healthy retina and Müller cell pathologies have detrimental effects on the entire retina.

In addition to the role of Müller cells in the GLO1 system discussed above, previous studies have outlined multiple DR pathologies which are in direct or indirect association with Müller cells (reviewed in [28, 29]). Müller glial cells become activated as one of the early pathogenic events of diabetes. Müller glial cells' activation has been characterized by the significant increase in expression of the intermediate filament glial fibrillary acidic protein (GFAP) in diabetic animals [30]. The ability of Müller cells in converting glutamate to glutamine is also reduced, resulting in significant increase of retinal glutamate early in diabetes progression [31, 32]. Müller cells are induced by hypoxic or glucose-deprived conditions to increase their production and secretion of VEGF [33]. Subsequently, the role of Müller cells in maintaining the BRB is significantly impaired and results in the increased vascular permeability observed in DR.

Aside from vascular leakage, glial cells' edema in diabetic retina is also thought to be due to impairment of potassium channels expressed by Müller cells [34]. One important function of Müller cells is to take up excess potassium ions, released from activated neurons, to prevent potassium-induced neuronal hyper-excitation and possible subsequent neurotransmitter and glutamate toxicity [35]. In diabetic retinae, the expression of inwardly rectifying potassium channel Kir4.1 is reduced and dislocated from its prominent expression sites near blood vessels, impairing the proper discharge of potassium ions from Müller cells into the blood and the vitreous [36]. Since the expression of channels which take up potassium ions from the extracellular space into Müller cells are unaffected, potassium ion concentration and osmotic pressure is increased inside the Müller cells of diabetic retina [37]. Thus, downregulation of the Kir4.1 channel in the retina of diabetic animals is associated with the swelling of Müller cells [38].

Another source of Müller cell edema in diabetic retina has been traced back to oxidative stress and mitochondrial dysfunction [39]. Müller cells in diabetic animals have shown to be more susceptible to osmotic stress compared to healthy animals [40]. Additionally, pathogenesis of DR is considered to be associated with elevated levels of

oxidative-nitrosative stress [12, 41]. Using various pharmacological blockers, Krügel et al. [39] demonstrated that swelling of Müller cell somata in diabetic rat retinae is predominantly linked to the oxidative stress due to production of ROS by xanthine oxidase enzyme. Moreover, they illustrate that mitochondria permeability transition is also a critical contributing factor in inducing mechanical stress and the subsequent Müller cell edema [39]. Lastly, inflammatory mediators are also suggested to play a role in Müller cell edema. Cyclooxygenase-2 for instance, is an inflammatory-related enzyme that is significantly upregulated in Müller glial cells of diabetic animal models [42].

III. Glucocorticoid Receptors

Glucocorticoid receptor (GR) is a nuclear receptor protein functioning as a ligand-dependent transcription factor which facilitates various actions of glucocorticoid hormones [43]. The inactive form of GR, encoded by the *Nr3c1* gene, is located in the cytoplasm as an oligomeric complex with molecular chaperones to maintain high-affinity for glucocorticoid hormones [44]. Once glucocorticoid ligands or agonists bind to the complex, it allows the receptor to dissociate from the regulatory complex and become hyperphosphorylated at multiple sites [45] (Fig. 1A). The activated GR then dimerizes in order to translocate to the nucleus and influence transcription (Fig. 1A). Glucocorticoids are steroid hormones produced by the adrenal cortex and regulated by the Hypothalamus-Pituitary-Adrenal cortex (HPA) axis. The endogenous ligand binding to GR in humans is called cortisol (corticosterone in mice), which is the primary regulator of inflammatory responses [46]. In case of diabetic patients, the HPA axis is found to be dysregulated leading to a chronic increase of cortisol levels, detected via urinary-free cortisol (UFC) outputs [47, 48]. Additionally, a positive correlation is reported between the degree of cortisol secretion and number of diabetic complications, including DR [47, 48]. Similarly, corticosterone levels in both mouse models of type 1 and type 2 diabetes are found to be significantly elevated [49]. Persistent activation of GR, whether by endogenous ligands or exogenous agonists, has shown to constitutively downregulate its own expression via an auto-regulatory loop [50]. Chronic activation of GR may therefore cause glucocorticoid resistance, impairing its beneficial anti-inflammatory role [45, 50].

Within the retina, modulation of glucocorticoid signaling via manipulating endogenous ligands or introducing artificial agonists has proven to be effective in various inflammatory diseases, DR included, as it efficiently counterbalances typical changes associated with diabetic retinopathy such as breakdown of the blood retinal barrier or onset of neuroinflammation (Fig. 1B). Gallina et al. [52] studied the role of GR signaling in retinal survival and microglial reactivity, using the chick retina as an *in vivo* model system. Primarily, they found that microglial reactivity was reduced after intraocular injection of dexamethasone as a GR agonist and increased by RU486 as a GR-antagonist. They further explained that GR activation suppresses microglial reactivity and prevents the loss of retinal function due to excitotoxicity. Hence, it seems as if activation of GR in Müller cells may have a protective role for retinal neurons. In another study performed by the same research group, activation of GR signaling in chick retina inhibited the formation of Müller glia-derived progenitor cells (MGPCs), while the inhibition of GR signaling induces the proliferation of MGPCs [44].

Shen et al. [53] further elucidated the role of glucocorticoids by investigating the effect of glucocorticoid treatment on neural and vascular pathology in Müller cell ablated mouse models. In this study, triamcinolone acetonide (TA) was used as a selective GR agonist to counter the effects of patchy Müller cell ablation in a transgenic model. Müller cell ablation causes photoreceptor degeneration, vascular leak and intra-retinal neovascularization, all of which are also observed in DR. Although loss of vision is ultimately due to photoreceptor degeneration, disruptions in the BRB and reactive neovascularization are key contributing factors in damaging photoreceptors. Hence, the most effective treatment approach must target affected neurons and blood vessels simultaneously. Previous studies have also shown that TA reduces vascular leakage [54], inhibits the secretion of VEGF [55] and prevents osmotic swelling of Müller cells [40]. Shen et al. [56] demonstrates that transgenic mice receiving the TA injections 4 days prior to conditional Müller cell ablation have significantly higher Müller cell survival. In line with that, GFAP immune-reactive activation of the surviving Müller cells was also significantly suppressed in the treated group compared to controls.

Taken together, it is quite established that glucocorticoid signaling impairment of retinal Müller cells plays a significant role in DR-related pathomechanisms. Moreover,

introducing GR agonists such as TA have been relatively successful in diminishing the detrimental effects of DR. An alternative and potentially more effective approach, however, may be the modulation of the GR expression itself as opposed to alteration in the concentration of available ligands or agonists. Upregulating the expression of GR induces downstream transcriptional events, resulting in enhanced glucocorticoid signaling. Thus, increased availability of the receptors will likely compensate for the absence of sufficient agonists in a more effective and enduring fashion. GR expression has shown to be almost exclusive to Müller glia in a highly conserved manner amongst warm-blooded vertebrates (i.e. retinae of chicks, mice, guinea pigs, dogs and humans) [44], making it a promising target for treatment development. Additionally, given the anti-inflammatory role of glucocorticoid signaling, GR is likely to be involved in DR pathomechanisms from early stages of disease. One possible explanation for downregulation of GR expression with progression of disease is the autoregulatory effects as a result of GR over-activation. Since other transcription factors involved in the glucocorticoid signaling pathway are downstream from GR, modulating the expression of GR may be most effective at early stages of disease. Howsoever, more experimental data are needed to unequivocally link the expression changes of GR to the progression of DR.

IV. Other Key Transcription Factors

Downstream of the glucocorticoid signaling pathway, there are multiple transcription factors that are pivotal to the anti-inflammatory responses in various tissues. A major pathway for such anti-inflammatory effects is mediated by an inhibitory protein-protein interaction known as transrepression [57]. When activated GR translocates into the nucleus, it directly binds to the JUN subunit of activating protein-1 (AP-1) and disrupts its transcriptional activation [58] (Fig. 1A). The AP-1 nuclear transcription factor is a heterodimer structure composed of c-JUN and c-FOS proteins [59]. Functionally, AP-1 is involved in a variety of cellular processes including proliferation, differentiation, apoptosis and pro-inflammatory responses [59] (Tab. 1). Therefore, transrepression of the AP-1 transcription factors by GR is one suggested pathway underlying the anti-inflammatory effects of glucocorticoid signaling. Given that Müller cells do express high levels of GR as

well as c-FOS and c-JUN (Fig. 2), it seems likely that GR could act via modulating transcriptional activity at AP-1 sites.

GR interacting protein (GRIP1) is a Müller cell-specific transcription factor (Fig. 2) involved in anti-inflammatory effects of glucocorticoid signaling. Based on the observed correlation between glucocorticoid repression and the recruitment of GRIP1 by AP-1 site reported by Rogatski et al., GRIP1 is suggested to be involved in GC-mediated repression of pro-inflammatory genes [60] (Fig. 1A). Furthermore, using *Grip1* knockout mice, Chinenov et al. illustrate the protective role of GRIP1 against systemic exaggerated inflammatory responses *in vivo* [61] (Tab. 1). The significant increase in serum levels of different cytokines in *Grip1*-deficient mice indicates the failure in restraining the production of pro-inflammatory cytokines. Hence, available evidence implies that GRIP1 plays a key role as a GR corepressor which facilitates the binding of activated GR to the AP-1 site in order to enable the anti-inflammatory effects of glucocorticoids.

Nuclear factor- *k*B (NF-*k*B) is a protein complex consisting of multiple transcription factors that are involved in cellular response to stress [62]. NF-*k*B signaling is fundamental in pro-inflammatory responses, though its hyperactivation has been implicated in the pathogenesis of several inflammatory diseases (i.e. diabetes) [63]. One of the key transcription factors in this complex is p65, encoded by the *Rela* gene [64]. Previous studies have shown that activating the glucocorticoid signaling pathway induces GR binding to the p65 transcription factor which results in: a) reduced NF-*k*B-mediated transcription, and b) hindered nuclear localization of p65 [62] (Tab. 1). In addition to the previously reported essential role of p65 in the anti-inflammatory pathway of glucocorticoid signaling, we could detect a highly Müller cell-specific expression of the *Rela* gene encoding this transcription factor, pointing to *Rela* as another promising gene of interest (Fig. 2B).

Transforming growth factor-β (TGF-β) signaling is another pathway which is influenced by glucocorticoid signaling. TGF-β signals, mainly known to function as regulators of cell proliferation [65], are transduced from the plasma membrane to the nucleus where they induce gene expression alterations [66]. Interestingly, previous research has shown that a source of TGF-β secretion in the retina is the Müller cells [67]. In addition, TGF-β signaling has been implicated in early pathogenesis of DR in studies

where effective drugs in suppression of experimental DR also suppressed the upregulation of the genes involved in TGF-β pathway [68]. The downstream effects of TGF-β signaling are well orchestrated through a family of similarly structured proteins referred to as Smads [66]. Pertinently, SMAD4 is one of the key factors in this pathway which was also found to be specifically expressed in Müller cells in our screen of transcription factors involved in glucocorticoid signaling (Fig. 2B). Song et al. illustrated the link between the glucocorticoid and TGF-β signaling pathways by demonstrating that GR specifically inhibits *Smad3* and *Smad4* transcriptional activity via the c-terminal activation domains [69]. Given the Müller cell specific expression of *Smad4* and its involvement in both TGF-β and glucocorticoid signaling pathways, SMAD4 may also be a potential transcription factor of interest in DR.

The *Stat3* gene coding for signal transducer and activator of transcription 3 (STAT3) is another gene that we found to be abundantly expressed in Müller cells of mouse retina (Fig. 2B). As mentioned before, vascular inflammation and elevation in pro-inflammatory cytokine levels are features of diabetic retinopathy which lead to fluid leakage and macular edema. Believed to be a facilitator of such response, STAT3 was identified as a transcription factor mediating cytokine signaling during vascular inflammation [70]. Yun et al. demonstrated that *Stat3* activation in retinae of mice reduces the expression of the tight junction proteins zona occludens-I (ZO-I) and occludin, leading to induced retinal endothelial permeability and vascular leakage [71]. Intriguingly, GR tethering to DNA-bound STAT3 is also reported to result in transcriptional repression of *Stat3* [72]. Hence, the extensive transcriptional interaction between GR and STAT3 is another piece of the glucocorticoid signaling pathway that may be a potential area of interest in understanding and treating DR pathomechanisms.

V. Gene Therapy

There are several methods currently being used for treatment and halting the progression of complications resulting from DR. These approaches include laser photocoagulation, injection of intravitreal corticosteroids and injection of anti-VEGF agents [76]. Although current treatment possibilities have shown to be relatively efficacious, they are associated

with major side effects and alternatives need to be explored. Laser photocoagulation has been reported to reduce contrast sensitivity and impair color perception due to its destructive approach [77]. Intraocular injection of corticosteroids such as TA or dexamethasone, used to impede inflammation and decrease capillary permeability, are also associated with complications such as increasing intra-ocular pressure, hemorrhage, endophthalmitis and increased incidence of cataract [78]. Likewise, long-term anti-VEGF therapy is believed to cause neurodegeneration and atrophy of the capillary network in the retina [79, 80]. Moreover, all the mentioned therapies rely on repeated injections which are costly and carry the risk of injection-related complications.

A potential novel therapeutic alternative to the available treatment options for DR may be achieved by using viral vectors for gene therapy. One of the first and most successful attempts to employ ocular gene therapy in humans has been approved for retinal degeneration treatment in patients with Leber's congenital amaurosis 2 (LCA2) [81]. Subretinal injection of adeno-associated viruses (AAVs) was illustrated to be safe and efficacious in replacing the defective RPE65 gene in LCA patients' retinal pigmented epithelium (RPE) [82]. Although not yet tested in human clinical trials to treat DR, gene therapy has indications of a promising treatment approach to replace or supplement the conventional possibilities for multiple reasons. Primarily, the effects of treatment last longer with gene therapy and repetitive injections are not necessary. Thus, the administration of treatment is associated with significantly fewer procedure-related complications and side effects. Additionally, gene therapy provides the opportunity to intervene at an earlier stage of disease as it offers preventive and protective advantages. The eye has been widely selected as an ideal environment for gene therapy research. This is mainly due to the presence of the BRB diminishing the leakage of viral vectors into the systemic circulation, availability of animal models exhibiting relevant ocular disorders and the small and bounded structure of the eye which requires minor amounts of vector for access to almost all ocular tissues [83]. Several animal studies have successfully used viral vectors in rodents to target (i) existing neurovascularization, retinal angiogenesis and vascular hyper-permeability [84, 85, 86, 87] and (ii) retinal microvascular dysfunction in order to protect neurons from further degeneration [88, 89, 90]. Given the prominent role of glucocorticoid signaling in the complications associated with DR and the efficacy of

synthetic glucocorticoid agonists in decelerating the progression of DR, a novel approach would be to employ gene therapy in modulating GR. That being said, of course the ethical concerns associated with the use of gene therapy must be considered. Suggesting gene therapy in early stages of disease as means to prevent the irreversible damages of DR demands strong confidence in positive outcomes. For instance, preclinical trials must clearly address the extent to which enhanced GR signalling in Müller glia may lead to unwarranted side effects, especially considering the multiple signalling pathways (e.g. NF-κB, TGF-β, STAT, JUN) that could be affected by this therapeutic approach. Additionally, potential immune responses, insertional mutagenesis and large-scale production of viral particles for delivery of therapeutic genes are viable concerns that should be taken into account [91]. However, these challenges can be addressed through further research into the efficacy of proposed treatments and seeking alternative methods of delivery.

VI. Conclusion

Given the high prevalence and burden of disease on the patients suffering from DR, developing novel methods of efficacious treatment is crucial to tackle this debilitating disease. Previous extensive research has illustrated the critical association of Müller glial cells with the pathomechanisms of DR. One of the fundamental targets in these studies has been GR which is almost exclusively expressed in the Müller cells of the retina. Known to play a pivotal role in anti-inflammatory responses, modulation of this receptor through administration of agonists or antagonist has been recognized as an effective approach. Considering the effectiveness of glucocorticoid treatment and the remarkable progress in the use of gene therapy at a clinical level, the next logical step would be to modulate the expression of Müller cell GR using viral vectors as a potential treatment for DR-associated pathologies.

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Compliance with Ethical Standards

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Tables

Table1. Summary list of selected potential transcription factors interacting with the glucocorticoid signaling pathway

Transcription factor	Potential endogenous role	Possible interaction with glucocorticoid signaling pathway	Reference
AP-1 (c-Jun and c-Fos)	Proliferation, differentiation, apoptosis and pro-inflammatory responses	Transrepressed when activated GR binds to the c-JUN subunit	[58, 59]
GRIP	GC-mediated repression of pro-inflammatory genes	Acts as a GR corepressor which facilitates the binding of activated GR to the AP-1 site	[60, 61]
***Rela* (p65)**	Involved in cellular response to stress and NF-*k*B pro-inflammatory signaling	Activated GR binds to p65 resulting in reduced NF-*k*B-mediated transcription and hindered nuclear localization of p65	[62, 63, 64]
SMAD3/4	Key factors in downstream effects of TGF-β signaling pathway, mainly as regulators of cell proliferation	Activated GR specifically inhibits *Smad3* and *Smad4* transcriptional activity via the c-terminal activation domains	[65, 66, 67, 68, 69]
STAT3	Mediating elevation in pro-inflammatory cytokine levels during vascular inflammation and down-regulating the expression of retinal endothelial tight junction proteins	GR tethering to DNA-bound STAT3 results in transcriptional repression of *Stat3*	[70, 71, 72]

Figures legends

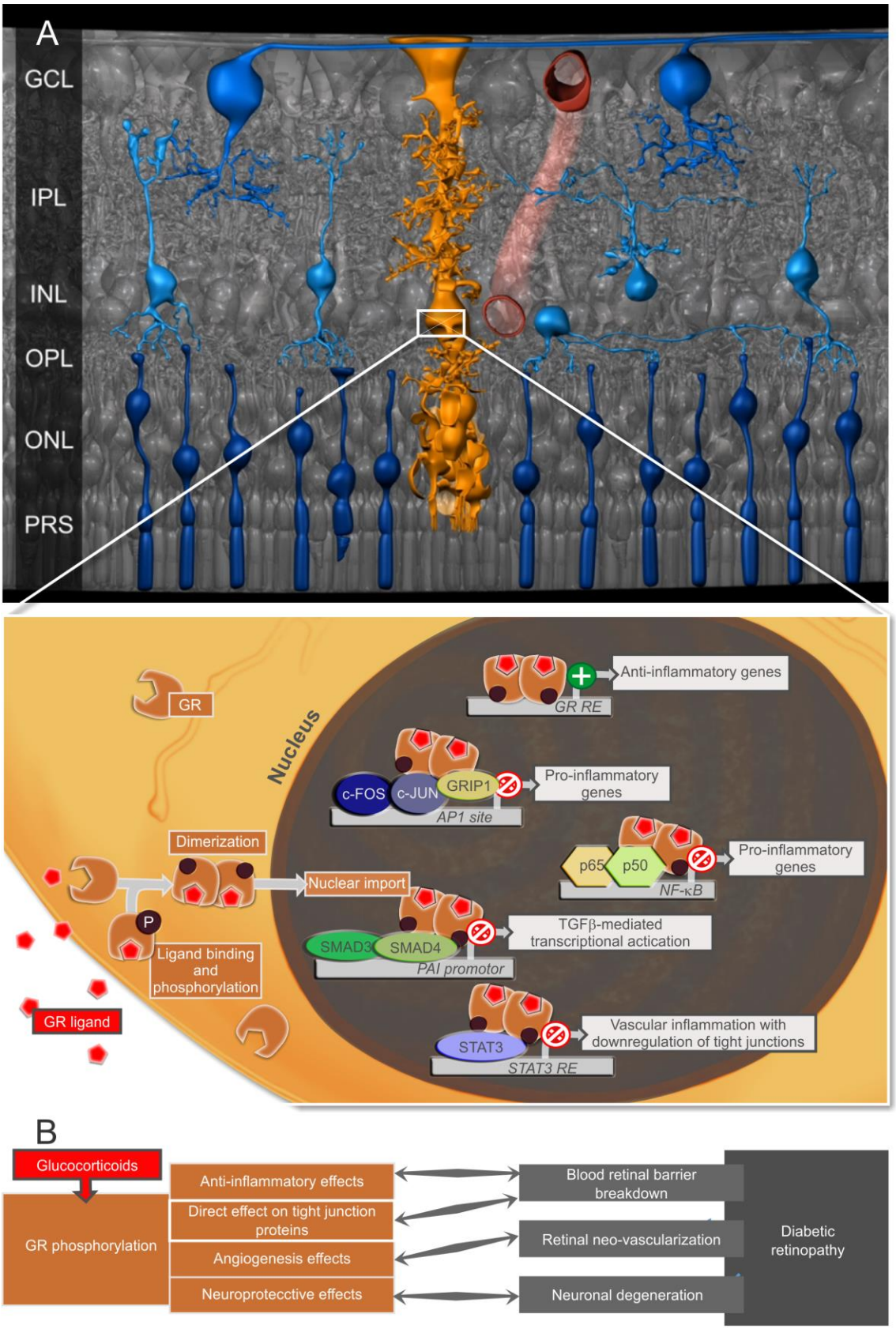

Figure 1. Glucocorticoid signaling in the retina – molecular basis and functional implications.

A *Top,* Müller cells (*orange*) contact every retinal cell type via multiple different specialized cell processes. Retinal neurons are highlighted in blue and endothelial cells in red. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium.

Bottom, schematic view on possible modes of action of the glucocorticoid receptor (GR) in Müller glia after ligand binding, receptor phosphorylation, dimerization and its translocation into the nucleus. Note that nuclear GR mediates its action via at least two different ways – either by binding to the glucocorticoid response elements (GR RE) in promotors of respective genes thereby activating their transcription or by tethering other transcription factors thereby repressing the expression of their respective target genes. P, phosphorylation.

B Visual summary of the opposing effects of glucocorticoid signaling and DR pathology on the retina. Modified after Zhang et al. [51].

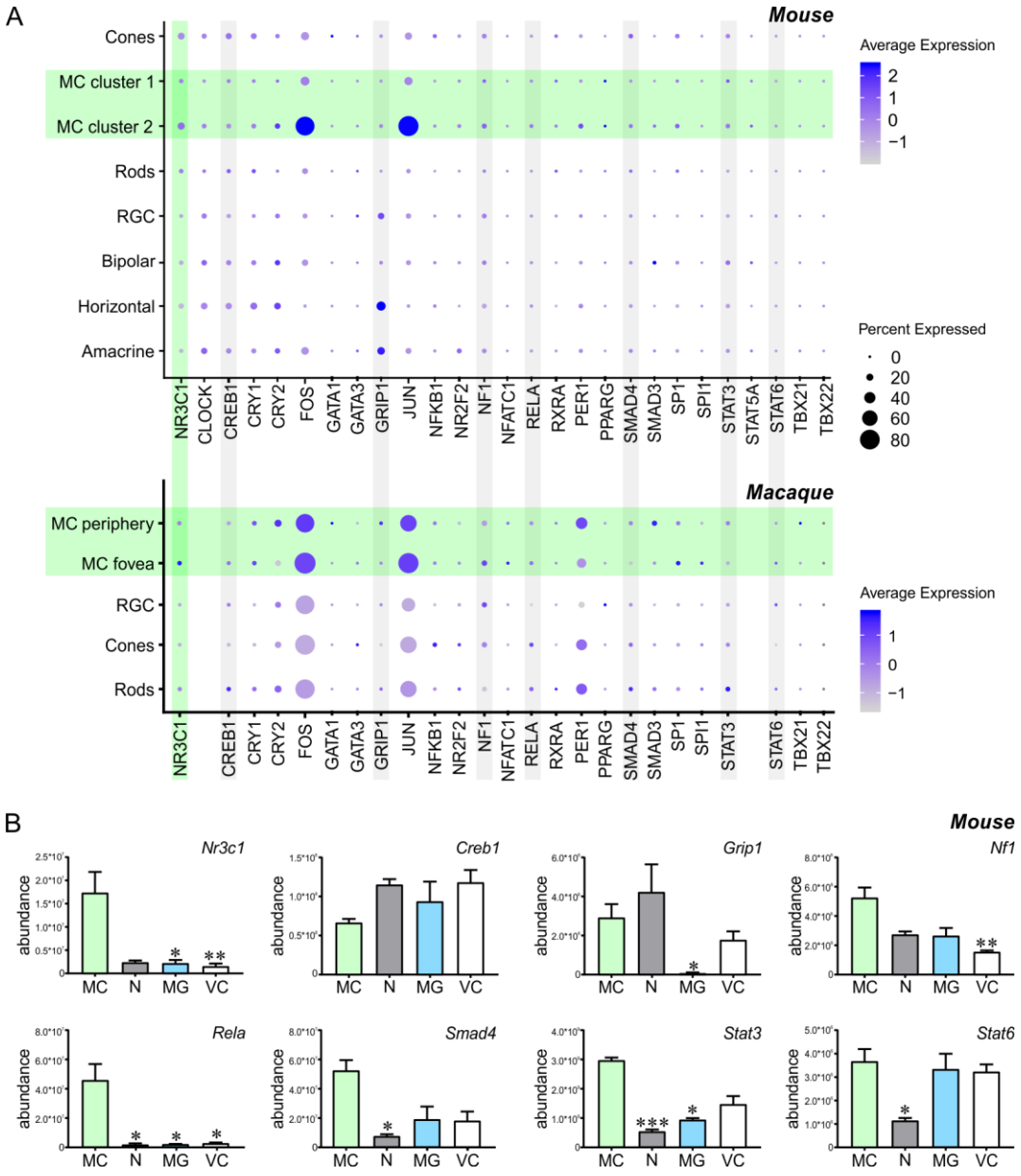

Figure 2. Expression of GR (Nr3c1) and interacting transcription factors in distinct retinal cell populations.

A Resource data of single cell RNA sequencing approaches on mouse [73] and macaque [74] retina were re-analyzed and the expression of putative GR-interacting transcription factors as identified by a literature research (table 1) was plotted. Note the highly Müller cell-specific expression of c-Jun and c-Fos. Even though most of the other described interactors were found to be expressed in Müller cells at transcript level, the difference in comparison to other retinal cell types was less

pronounced.

B Results form a screen of protein expression of retinal cell types purified using magnetic activated cell sorting from the adult, pigmented mouse retina via LC-MS/MS mass spectrometric analysis as described in Mages et al. [75]. It confirms Müller cell-specific expression of the glucocorticoid receptor (Nr3c1) and, interestingly, also identifies major differences in expression levels of GR-interacting transcription factors at the level of protein expression. GRIP1, RELA, SMAD4 and STAT3 are primarily expressed in Müller cells supporting the hypothesis of their contribution to GR signaling in Müller glia as suggested in Fig. 1A. Bar represent the mean ± SEM of protein abundance measured in 5 biological replicates. Comparison of protein expression level with that in Müller cells (tested via one-way-anova with correction for multiple testing): ***P<0.001; **P<0.01; *P<0.05. GR, glucocorticoid receptor; MC, Müller cells; N, retinal neurons; MG, microglia; VC, vascular cells.