

● PERSPECTIVE

## Diabetic retinopathy: a matter of retinal ganglion cell homeostasis

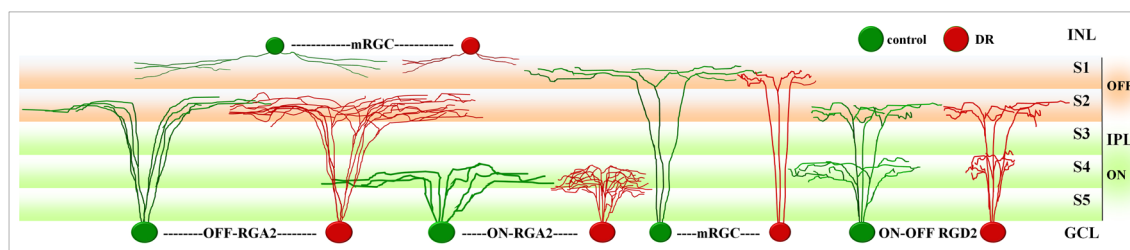
Diabetic retinopathy (DR) is the most common complication of diabetes and the leading cause of visual impairment in the working-age population in the Western world (<http://www.diabetesatlas.org/>). One of the earliest events observed in DR is the impairment of retinal perfusion, caused by the constriction of retinal blood vessels, inducing biochemical and metabolic alterations, pericytes/endothelial degeneration, and retina ischemia (Wong et al., 2016). Severe stages of DR include proliferative DR, caused by the abnormal growth of new vessels, and diabetic macular oedema in the central part of the retina. DR is strongly associated with a prolonged duration of diabetes, hypertension, and hyperglycaemia but it can persist even after replacement of normal glycaemic status (Wong et al., 2016). The fact that low-grade inflammation, particularly associated with the early stages of DR, induces structural and molecular alterations which play critical roles in the pathogenesis of DR is gaining attention in recent years (Mesquida et al., 2019). Although DR is traditionally regarded as a microvascular disease, retinal neurodegeneration is also involved. In particular, growing evidences indicate that the degeneration of retinal neurons also occur before clinical signs of DR and the typical microvascular alterations of DR may be a subsequent event (Wong et al., 2016; Simo et al., 2018). However, at present, there are no decisive data confirming this scenario since the timing of neuronal death and the rate of susceptibility of specific cell population during DR is still controversial. In this article, we will highlight the latest findings on the retinal ganglion cells (RGCs), the inner retinal heterogeneous neurons in which the cell death related to DR is detected first (Simo et al., 2018), with the aim to emphasize that the homeostasis of RGCs (or specific subpopulations of RGCs) could be a main issue to mitigate hyperglycaemic damage in the retina.

**DR affects RGCs:** The importance of RGC death/survival in diabetes and the characterization of RGCs suffering DR insult is a developing story, including therapies to inhibit the neurodegeneration, although critical information on the cellular and molecular events of DR dysfunction is yet needed. Recent evidences revealed that at early stage of diabetes, deep morphological changes and electrophysiological defects take place, mainly in the neurons of the inner retina (Sergeys et al., 2019). Indeed, diabetic mice showed a reduction of neural retina affecting, in particular, the ganglion cell complex, which includes nerve fiber layer, ganglion cell layer (GCL), and inner plexiform layer, and the nerve fiber layer-GCL complex. The inner retinal thinning and loss-of-function of inner retinal cells paralleled a significant decrease in RGC density and amacrine cells (Sergeys et al., 2019). These time-dependent events can occur at the early stage of DR and generally become more severe with the progression of diabetes.

By combining morphological, functional and genetic features, RGCs can be classified into at least forty distinct types, each of which covers the mammalian retina to encode reliably its part of the visual message (Christensen et al., 2019). The classification into RGC type A–D (RGA, RGB, RGC, and RGD) subgroups is morphologically based on the RGC soma sizes and dendritic characteristics. Functionally, RGCs can be defined ON- and OFF-center RGCs as they respond best to increases and decreases in light intensity delivered to their receptive field centers, while ON-OFF RGCs respond to both increases and decreases in light intensity. In diabetic mice, early stage of hyperglycaemia mainly altered the dendrites of RGCs arborizing in the ON sublamina of the inner plexiform layer, with less significant changes in OFF sublamina (Cui et al., 2019). The subset of RGCs ON-type RGA2 (ON-RGA2) displayed an appreciable reduction of the dendritic field areas while OFF-RGA2 cells were unaltered. In addition, an increase in total number of dendritic branches was detected both in ON- and OFF-RGA2 cells. Noteworthy, ON and OFF signaling and interactions in the visual system are closely involved in contrast detection. Electrophysiologically, only ON-RGA2 showed increased resting membrane potentials and decreased membrane capacitance during diabetes although both ON- and OFF-RGA2 displayed an enhanced excitability. This morphological and functional remodeling could be explained as an attempt to balance the loss of presynaptic inhibitory inputs, such as those generated by amacrine cells. In this respect, in non-pathological conditions, RGA2

cells (equivalent to  $\alpha$ -RGCs, the best recognized RGCs in mammalian retina with the biggest size of soma and branching) exhibit a large receptive field and show a short response latency/fast conducting axons, definitely playing an essential role in visual processing. Likewise, the directionally selective RGCs have a key role in the retina functions since they respond best to motion of a stimulus in a particular direction. In diabetic mice, the ON-OFF direction-selective RGD2 cells, arborizing both in ON and OFF sublaminae of inner plexiform layer, have a reduced branching in ON-stratified dendrites (Cui et al., 2019). On the contrary, the ON-direction selective cells RGC1, which are known to be resistant to pathological insults, were unaffected. Glutamate excitotoxicity, through the overstimulation of mainly alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate and N-methyl-D-aspartate (NMDA) receptors, which results in an uncontrolled intracellular  $Ca^{2+}$  response in postsynaptic neurons and cell death, is a key initial process in DR and RGCs loss (Simo et al., 2018). In the mammalian retina, alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate and NMDA receptors are expressed by all RGCs. It has been suggested recently that the early NMDA-induced insult may cause the reshape of RGCs dendrites by affecting synaptic activity before RGCs apoptosis takes place (Christensen et al., 2019). Noteworthy, a diverse susceptibility of different RGCs to NMDA excitotoxicity exists. Indeed, NMDA overstimulation in mice altered mostly the J-RGCs (an OFF-RGC population expressing the immunoglobulin superfamily recognition molecule named junctional adhesion molecule B) while  $\alpha$ RGCs being the most resistant type. Since the  $\alpha$ -RGCs subtype RGA2 are particularly damaged by diabetic insult (Cui et al., 2019), one may speculate that NMDA excitotoxicity is not a key event mediating DR-induced RGA2 death. The fact that NMDA overstimulation injured also BD- (ON-OFF RGCs sensitive to ventral motion) and W3-RGCs (the smallest in size and the most numerous RGCs, functionally uncharacterized) indicates that RGCs susceptibility is not correlated to the ON and OFF inputs, not even to their soma and dendritic field size (Christensen et al., 2019). Among RGCs subtypes, melanopsin-expressing RGCs (mRGCs) are intrinsically photosensitive neurons participating in “non-image forming” visual processing in the retina, likely performing functions also beyond circadian ones. In humans, mRGCs are characterized by large soma, located mainly in the GCL and in smaller numbers in the inner nuclear layer, and wide dendritic arbor (Obara et al., 2017). When compared with non-melanopsin RGCs, they seem to be more resistant to glutamate toxicity occurring in several pathologies (Obara et al., 2017). Although discrepancies have been reported in animal models, a relevant damage of mRGCs was recently detected in patients with severe DR phase, showing not only symptoms of visual impairment but also a compromised photo-entrainment of the circadian clock (as for instance circadian misalignment, pupillary defects, and sleep disorder) (Obara et al., 2017). In particular, the progression of DR correlates with a considerable loss of RGCs, including an extensive reduction of mRGCs. The loss of mRGCs is more pronounced in inner nuclear layer than GCL thus indicating a different damage among nuclear strata. These data further indicated that hyperglycaemia effects can be detected on RGCs as a key target and, of interest, these effects are cell subtype-dependent. The possibility that mRGCs loss might occur prior to neurodegeneration of conventional RGCs deserves to be investigated. The **Figure 1** summarizes DR-induced changes at dendritic field of distinct RGCs, although a systematic picture on the fate and morphology of RGC subtypes during hyperglycaemia is still to come.

DR is a multifactorial pathology and items that play multiple roles in degenerative cellular processes are taken into special consideration as possible targets for RGCs. Apoptosis participates in the death of retinal neurons under different conditions, including DR, but the catabolic pathway autophagy has been recognized also to be involved (Amato et al., 2018). Using *ex vivo* mouse retinas treated with high glucose, we have demonstrated recently that neurons which are committed to die by apoptosis exhibit an impairment of autophagic machinery, reflecting an altered equilibrium of apoptosis and autophagy in the early phases of DR (Amato et al., 2018). In particular, in several retinal cell populations of high glucose-treated explants, including RGCs, we observed an increase of caspase-3 activity and a significant accumulation of autophagosomes. The decrease of autophagic turnover being the consequence of mTOR (mammalian target of rapamycin) activation, a master regulator of autophagy. To note, retina treatment with octreotide, a well-known analogue of the neuropeptide somatostatin, preserved hyperglycaemic neurons from apoptosis and, concomitantly, increased robustly the autophagic activity inhibiting mTOR (Amato et al., 2018). These re-



**Figure 1** Major changes induced by DR affecting the dendritic field of different RGCs.

RGC branching pattern and branching level in the five strata (S1–S2: OFF sublaminae; S3–S5: ON sublaminae) of the inner plexiform layer (IPL) was depicted. The dendritic arborization of melanopsin-expressing RGCs (mRGCs) located in the inner nuclear layer (INL) was showed as well. DR: Diabetic retinopathy; GCL: ganglion cell layer; RGA: RGC type A; RGC: retinal ganglion cell; RGD: RGC type D.

sults suggest that somatostatin is an endogenous pro-survival system in DR acting, at least in part, as an autophagy-modulating factor. Different evidences support the neuroprotective role of neuropeptides in retinal diseases and re-balancing the dysfunctional cross-talk between apoptosis and autophagy generated by injurious conditions is considered a key weapon in their arsenal (Cervia et al., 2019). Accordingly, we have reported that apoptosis/autophagy equilibrium can be a fruitful target for preserving RGCs and retinal cells in other pathological conditions, as for instance oxygen-induced retinopathy (Cammalleri et al., 2017). In line with this hypothesis, histone HIST1H1C, that play a central role in the apoptosis machinery, was identified as a regulator of autophagy in DR (Wang et al., 2017). In particular, histone HIST1H1C overexpressed in the retina of diabetic mice and promoted aberrant autophagy maintaining the deacetylation status of H4K16. Histone HIST1H1C was responsible also for retinal inflammation, gliosis, and loss of RGCs, similar to the pathological changes identified in the early stage of DR. Its downregulation rescued the diabetic-induced hallmarks, restoring physiological autophagy and attenuating the death of RGCs (Wang et al., 2017). Confirming the coexistence of multiple processes that differently contribute to RGCs apoptosis in DR, a role of Brg1/Notch axis was observed recently (Zhang et al., 2019). Using *in vitro* RGCs administered with high glucose, authors showed that Brg1 expression decreased. Brg1 (the central catalytic subunit of chromatin-modifying enzymatic complexes) is a crucial modulator of gene expression, since it participates in the control of chromatin dynamics essential for physiological cellular processes. Brg1 upregulation enhanced the viability and reduced the apoptosis of diabetic RGCs thus suggesting an additional target to preserve RGCs during DR-induced damage. Of interest, it was observed that Brg1 protects RGCs by increasing the activation of the transmembrane receptor Notch and its pro-survival pathway, including the protein kinase Akt which is a well known regulator of autophagy (Zhang et al., 2019).

**Concluding remarks:** Since increasing evidences indicate that the RGCs loss is an early event in diabetes, a crucial point for therapies preventing DR is to avoid or mitigate retinal neurodegeneration. The regulatory balance between death/dysfunction and life/function in DR shifts as neuronal population changes. In this respect, further studies using neuroprotective tools should broaden their scope beyond neuronal death to include shape, dendritic arborization, intracellular pathways, and functions of RGCs. Indeed, morphological and physiological changes in RGCs determine their ability to process the photo-reception and, in a certain extent, these alterations are responsible of “image-forming” and non-image forming” visual defects occurring in DR. These are all together primary aspects, especially for the development of diagnostic tools and therapeutics approaches, which have the purpose to reduce retinal damage of diabetic patients.

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**Elisabetta Catalani\***, **Davide Cervia\***  
Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, largo

dell’Università snc, Viterbo, Italy  
**\*Correspondence to:** Elisabetta Catalani, PhD, [ecatalani@unitus.it](mailto:ecatalani@unitus.it);  
Davide Cervia, PhD, [d.cervia@unitus.it](mailto:d.cervia@unitus.it).  
**orcid:** 0000-0002-1210-6836 (Elisabetta Catalani)  
0000-0002-2727-9449 (Davide Cervia)  
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