Regulatory and Pathogenic Roles of Müller Glial Cells in Retinal Neovascular Processes and Their Potential for Retinal Regeneration

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Abstract
Müller glial cells are known to play a very important role in retinal homeostasis, and many metabolic functions have been ascribed to these cells not only in the normal but in the diseased retina. This chapter addresses a wide variety of activities attributed to Müller glial cells. It describes how activation of Müller glia by pro-inflammatory and pro-angiogenic factors may contribute to the development of neovascularisation and fibrosis, the characteristic pathological features of diabetic retinopathy. The chapter also highlights the regulatory role that Müller glia exert in various retinal functions, including the prevention of neural damage due to their production of neurotrophins in response to inflammatory and angiogenic signals. Recent findings that a sub-population of Müller glia have the ability to regenerate retinal neurons in adult life are described, and the implications for the potential use of Müller stem cells for cell-based therapies to regenerate diabetic retina are discussed.

Müller cells constitute the major glial cell population of the retina. They expand across the whole width of the retina and are in direct contact with all retinal cell types. They stabilize the complex retinal architecture, provide structural support to retinal neurons and blood vessels, and prevent aberrant photoreceptor migration into the subretinal space [1]. The mammalian retina harbours between $10^6$ to $10^7$ Müller cells [2] and their morphology varies according to their location within the retina. Müller glia in the retinal periphery comprise short thick cells with big end feet, whereas the thicker central retina is occupied by long slender cells with small end feet [2]. Independent of their location, Müller glia are sensitive to their environment and adapt to local surroundings. Within the nuclear layers of the retina, they ensheath local neurons and within the plexiform layers they extend out numerous cytoplasmic processes.

Müller glial cells promote neural survival in the retina due to their ability to remove metabolic waste from the retina and to produce trophic factors. They perform functions that astrocytes, oligodendrocytes and ependymal cells effect in other regions of the central nervous system [1]. In vitro, Müller cells promote extensive neurite outgrowth from rods [3], express several neurotransmitter receptors, including γ-aminobutyric acid type B receptors [4], and various types of glutamate transporters which facilitate glutamate uptake in order to keep its extracellular concentration below neurotoxic levels. Müller cells also express...
glutamine synthetase, an enzyme involved in detoxification of ammonia and glutamate that operates in concert with the L-glutamate/L-aspartate transporter to terminate the neurotransmitter action of glutamate [5]. They express K⁺ channels on their plasma membrane, specially inwardly rectifying K⁺ (Kir) channels, that makes them highly permeable to K⁺ [6].

Changes in Müller cell membrane conductance have been extensively reported in proliferative diabetic retinopathy (PDR). It has been suggested that downregulation of active Kir channels and membrane depolarization are likely to disturb voltage-dependent Müller cell functions, such as regulation of local ion concentrations and uptake of neurotransmitters. In addition, it has been thought that enhanced entry of calcium ions from the extracellular space and the subsequent stimulation of calcium-activated potassium channels may support the Müller cell proliferation observed in this complication of diabetes mellitus [7].

In addition to the above metabolic functions, Müller cells are known to produce multiple cytokines, growth factors and neurotrophic factors in response to inflammatory and angiogenic signals, retinal hypoxia and advanced glycation end products [7]. Although the metabolic and structural functions of Müller glia in the adult eye have been known for a long time, it was not until recently that a new function for these cells as a source of retinal neurons was identified in the adult mammalian eye, including humans [8–10].

There is considerable evidence that neural degeneration observed in the diabetic retina is due to reactive changes in Müller glial cells. Neural cell damage and death have been observed prior to the onset of neovascularisation in animal models of diabetic retinopathy (DR), and accumulating evidence shows that during the diabetic process, Müller cells become gliotic and display altered potassium siphoning, glutamate and γ-aminobutyric acid uptake and express various modulators of angiogenesis [11]. Müller glia have been thought to operate as a means of communication between the neural retina and the vasculature. Microscopic studies of primate retinæ have demonstrated that the Müller cell end feet completely encircle the retinal blood vessels [12]. This close anatomical apposition suggests that Müller glia form an essential part of the blood retinal barrier and changes in this structure caused by Müller cell damage are believed to contribute to vessel leakage in DR.

Role of Müller Glia in Inflammation and Angiogenesis

Several studies have implicated inflammation as a contributing factor in the microvascular and glial changes observed in DR, a common ocular complication in patients with diabetes mellitus. This evidence is derived from studies that show that fibrovascular membranes from eyes with PDR contain high levels of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, monocyte chemotactic protein-1, and macrophage colony-stimulating factor [13–15].

Vascular endothelial growth factor (VEGF), a potent pro-angiogenic mediator which has been implicated in the pathogenesis of DR [16], has been shown to be present in the vitreous of patients with PDR, along with another pro-angiogenic factor, the hepatocyte growth factor [17]. Levels of these two factors in the vitreous of diabetic patients have been shown to correlate with the severity of the condition. Müller glial cells are thought to play an important role in the pathogenesis of this complication as they have been shown to produce most factors found in the vitreous of diabetic patients complicated by PDR. Advanced glycation end products are known to induce production of IL-6 [18] and VEGF [19] by Müller cells in vitro, whilst pro-inflammatory cytokines such as IL-1 can also activate these cells to release IL-6 [20]. High glucose levels induce production of TNF-α and IL-1β by Müller
Fig. 1. Multiple metabolic and cellular functions of Müller glial cells. Illustration of the multiple functions and cell-ECM interactions of Müller glia that lead to activation of several pathways and to the release of pro-angiogenic, anti-angiogenic and neuroprotective factors implicated in the pathogenesis of DR. HB-EGF = Heparin-binding epidermal growth factor-like growth factor; MCP-1 = monocyte chemotactic protein; HGF = hepatocyte growth factor.

Glia in culture [21], whilst activation of these cells with lipopolysaccharide and interferon-γ promotes release of TNF-α by these cells [22]. There is also evidence that human Müller cells express the isoform variant VEGF₁₈₃ [23] and that gene upregulation and production of VEGF by these cells may be induced by hypoxia [24], bFGF [25] and heparin-binding epidermal growth factor-like growth factor [26]. In addition to their production of pro-angiogenic factors, Müller cells have been shown to release an important anti-angiogenic factor known as pigment epithelium-derived factor (PEDF) [27]. This is of special interest as it has been recognized that the balance between VEGF and PEDF and their reciprocal interaction are important for the regulation of vascular permeability and angiogenesis. PEDF has been demonstrated to significantly decrease VEGF expression by Müller cells, whilst silencing of the PEDF gene by siRNA in Müller cells has resulted in significant upregulation of VEGF expression at both RNA and protein levels [27]. These observations suggest that PEDF is an endogenous negative regulator of VEGF in Müller cells, and that regulatory Müller glial functions are important for adjusting the balance between pro-angiogenic and anti-angiogenic mediators during retinal neovascular processes that characterise the development of DR.

Figure 1 attempts to summarize the major events that lead to activation of Müller glia within the diabetic retina, and the contribution of various factors released by Müller cells to the activation of cellular functions responsible for neovascularization, fibrosis and neural survival.

Control of Extracellular Matrix Deposition by Müller Cells

Early events leading to the development of PDR involve local cell migration and proliferation followed by extracellular matrix (ECM) deposition [28]. These cellular processes eventually lead to the formation of new vessels and fibrovascular complexes. Cell migration and matrix deposition are controlled by ECM degradation by proteolytic enzymes known as matrix metalloproteinases (MMPs) [29]. These enzymes, also known as matrixins, constitute a family of zinc-binding,
calcium-dependent molecules, whose activity is regulated by natural inhibitors known as tissue inhibitors of metalloproteinases (TIMPs) [29].

Two major matrix degrading enzymes, known as MMP-2 (collagenase A) and MMP-9 (collagenase B) are found in the vitreous of eyes with PDR [30], whilst characteristic staining for active MMP-9 has been observed within the perivascular matrix of neovascular membranes with this condition [31]. The inactive forms of MMP-2 and MMP-9 have also been shown to be significantly elevated in the neovascular membranes in comparison with normal retinas [32]. Although the main source of these two MMPs in vivo has been thought to be the retinal pigment epithelial cells, which are well known to produce these molecules in vitro [33], evidence has been presented that Müller cells produce both MMP-2 and MMP-9 in vitro, and that cytokines such as TNF-α either in soluble form or bound to the ECM may induce upregulation of MMP-9 expression by these cells [34].

Despite extensive studies demonstrating the production of several MMPs and TIMPs by retinal pigment epithelial cells, there are very few investigations on the production of TIMPs by Müller glia. At present, there is limited evidence for the Müller cell expression of TIMP-2 in vivo, as demonstrated by studies showing Müller glia staining with antibodies to TIMP-2 within the degenerating retina of the rd1 mouse [35]. At the intracellular level, Müller cells have been shown to contain abundant mitochondria which are reflective of their active metabolic role within the retina. It is of interest that MMP-1, which has been shown to associate with mitochondria and to protect cells from apoptosis, is abundant within these organelles in Müller cells [36].

That Müller cells have the ability to release MMPs that promote the degradation of ECM, together with the evidence that MMPs promote cell migration and proliferation, strongly suggests that these cells play a very important role in the control of cell-ECM interactions that lead to the development of retinal neovascular processes.

**Neuroprotective Role of Müller Glia**

As part of their neuroprotective role within the retina, Müller glia produce a group of proteins that signal for neural survival, growth and differentiation. These proteins, known as neurotrophins (NTs), constitute a group of four structurally related factors known as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT3 and NT4. NTs effect their functions by binding to two different surface receptors: the tropomyosin-related kinase receptors and the p75 NT receptor (p75NTR). NT binding to their receptors leads to phosphorylation of tyrosine residues in the receptor cytoplasmic domain. This causes activation of signalling pathways such as Ras, Rap and PI3K that in turn lead to the induction of cell survival and neurite outgrowth [37]. BDNF has been recognized to play an important role in the development, differentiation, connectivity and survival of retinal neurons [38], and Müller glial cells have been shown to constitute an important source of BDNF in vivo [39] and in vitro [40, 41]. There is evidence that BDNF promotes photoreceptor survival following experimental retinal detachment [42], and that it exerts trophic and neuroprotective effects on retinal ganglion cells during development and after optic nerve injury [43]. In addition, BDNF can rescue photoreceptors from the damaging effects of constant light, protect the retina from ischaemic injury, and modulate the morphology and the neurochemical phenotypes of amacrine cells [44]. Neural cell damage induced by cytotoxic factors such as glutamate, result in an enhanced production of BDNF by Müller glia [41] and intravitreal injection of BDNF in eyes from rodents susceptible to retinal degeneration increases expression of NT receptors on Müller glia but not on photoreceptors, although there is increased photoreceptor survival, suggesting that neurotrophic factors act indirectly through Müller cells to promote this phenomenon [45]. In addition, BDNF causes activation of intracellular