

# Paving the way toward retinal regeneration with mesencephalic astrocyte-derived neurotrophic factor

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Neurodegenerative diseases are a leading cause of disability worldwide, and despite significant resources put toward the discovery of potential therapeutic targets, there are currently no effective treatments. The rise of methods to derive and propagate stem cells *in vitro* offered great promise toward the development of cell replacement therapies. Unfortunately, these attempted therapies were stymied by extremely low cell survival and a lack of integration into the pre-existing circuitry due, in part, to an unfavorable environment. A recent report in *Science* from the laboratories of Heinrich Jasper and Deepak Lamba at the Buck Institute by Neves *et al.* (1) provides evidence that components of the immune system may underlie the favorability of a tissue microenvironment to cell survival and regeneration. Furthermore, they identify mesencephalic astrocyte-derived neurotrophic factor (MANF) as a potential mediator of regenerative favorability.

Efforts to boost the intrinsic regenerative potential of neurons in the central nervous system (CNS) revealed the importance of a permissive environment for successful regeneration (2). Consequently, extensive research has been devoted toward the understanding of both the cell intrinsic and environmental factors required to facilitate regeneration as well as methods to coax them toward a more permissive state. While we are still far from being able to regenerate damaged neural tissue, progress has been made in uncovering and manipulating intrinsic factors, largely due to advances in stem cell biology (3).

In contrast, modulation of the environment has been an inherently more complicated task. In the CNS, glial and immune cell types are major players in secreting factors into the local neural environment, many of which can have opposing effects, leading to an environmental milieu containing both regenerative and non-regenerative factors (4). In the context of damage, neurons will release distress factors which serve to recruit and activate local glial and resident immune cells. Activation prepares glia and macrophages for the clearance of cellular debris derived from the damaged neurons. Unfortunately, activation is often accompanied by an upregulation of inflammatory cytokines and other non-regenerative factors (5).

Although it is necessary for dying neurons to be removed, the mechanisms which facilitate clearance often also exacerbate damage, leading to further cell death and the creation of an environment that is hostile to the replacement of the damaged cells (6). The ultimate goal, then, is to find a way to modulate the activation response so it involves only the features that are conducive to regeneration. Progress toward this goal has been facilitated by the recognition that reactive macrophages, microglia, and astrocytes can exist in different states. These activation states are broadly defined as pro- and anti-inflammatory, and are classified as M1 and M2, respectively (7). Presumably then, the most useful therapies will be those which strategically shift the balance in favor of the M2 state. Toward this goal, Neves *et al.* have potentially made a major breakthrough in the ability

to influence immune cell activation state and regenerative permissiveness in the mammalian retina using MANF.

MANF is an endoplasmic reticulum (ER) stress protein which can be localized to the ER lumen or secreted as a neurotrophic factor, suggesting it may play both intracellular and autocrine/paracrine functions (8). It is primarily understood to be involved in the unfolded protein response, regulated by GRP78/BiP to protect against ER stress induced cell death (9). MANF was originally found to be neuroprotective in dopaminergic neurons (10) and in the context of CNS ischemia (11,12). Since MANF is expressed in neurons, its intracellular role as an anti-apoptotic agent has been thought to underlie its neuroprotective effects (13). Although a role for MANF within photoreceptors cannot be ruled out, the work of Neves *et al.* reveals that, in the retina, the primary neuroprotective effect of MANF is derived from its effects on immune cells, rather than a direct effect on neurons. Another group has demonstrated that MANF is expressed only in activated subsets of microglia during ischemia (14) and macrophages in the spleen (15). This supports the idea that MANF is involved in immune cell regulation more broadly, though studies specifically addressing the generalizability of MANF's immune modulation are still needed.

Neves *et al.* show that immune cells provide both a source and a target for MANF, suggesting that it is working in an autocrine fashion to alter its own state, and/or in a paracrine manner to bias the activation state of its neighbors. Furthermore, they demonstrate that MANF loses its neuroprotective abilities in the retina when the immune cells are prevented from entering the M2 state. Although they provide convincing evidence that MANF can promote a regenerative environment, the mechanism is still unclear. The most critical question is how MANF interacts with its (unidentified) receptor and the signaling pathways affected by this interaction. The KDEL receptor (KDELr) has been implicated as a potential MANF receptor, both intra- and extracellularly (16). The work by Neves *et al.* indicates that MANF is working through KDELr in drosophila hemocytes (innate immune cells), yet whether this finding translates to mammalian immune cells has yet to be determined. Although the mechanism of action for KDELrs is also unclear, they are known to affect G-protein coupled receptors and membrane receptor clustering, triggering downstream signaling cascades (17). Characterization of the interaction between MANF and its receptor will help answer these questions.

Based on the work by Neves *et al.*, efforts directed

toward uncovering the molecular mechanisms involved in the MANF signaling should be an active area of research. It has become clear in recent years that the characterization of activation states into two broad classes is an oversimplification (18), and a more realistic picture likely involves a spectrum where only a subset of the 'alternatively activated' cells are truly pro-regenerative. MANF may be selectively inducing a special M2 subclass, and it will be important to further characterize the immune cells in MANF treated retina beyond the expression of a few basic M2 markers. Ultimately, a better understanding of the mechanisms involved in establishing immune cell activation states, particularly M2, may have tremendous benefits in the production of future therapeutic targets.

Neves *et al.* indicate that the neuroprotective effect of MANF is linked to the activation of PDGFR $\alpha$ . Although they show that MANF can be induced through the action of PDGF-A and its receptor PDGFR $\alpha$ , the cell type mediating the direct effects remains unclear. The authors presume that PDGF-A is acting directly on the immune cells, and ignore the likely possibility that it is instead (or also) acting on the Muller glia. Muller glia are retinal radial-glia like cells which express PDGFR $\alpha$ , can become activated in response to neuronal distress signals, and have multipotent stem cell potential that facilitates retina regeneration in some species (19). Neves *et al.* indicate that MANF is also localized to Muller glial processes following photoreceptor damage, but fail to elaborate on how Muller glia may be involved in the neuroprotective actions of MANF. Similar to macrophages, MANF is only expressed at appreciable levels in glia in response to stress or damage (14). Glial cells can also have a spectrum of responses ranging from neuroprotective to pro-apoptotic in the damaged CNS, yet little is known about how glial activation states are established and regulated (20). Communication between glial and immune cells is known to influence the activation state of both cell classes (21), thus the signaling between them may also determine the overall inflammatory state of the area in which they reside. The interplay between Muller glia and immune cells may be critical to the neuroprotective and possibly even the M2 response of MANF in the retina. A complete understanding of the mechanisms underlying MANF's effects cannot be achieved without also investigating the role of MANF in Muller glia.

The clinical implications from the work of Neves *et al.* are significant. MANF appears to be able to protect photoreceptors from dying in a mouse model of retinal

degeneration, as well as enhance the long-term survival and integration of subretinally injected donor photoreceptors into degenerating retinas. Therefore, as a therapeutic agent, MANF can both delay the progression of degeneration and facilitate retina regeneration. It will be interesting to see if MANF also provides an advantage when combined with therapies aimed at enhancing the intrinsic regenerative potential of the retina itself, particularly those targeting Muller glia.

It is worth noting that the authors of this study are funded by Amarantus Biosciences, Inc., which was co-founded by one of the investigators who discovered the neuroprotective effect of MANF in dopaminergic neurons (10). Amarantus has decided to validate the clinical neuroprotective efficacy of MANF in the retina, and has received orphan drug designation from the European Union for the treatment of retinitis pigmentosa (RP) as well as the United States for the treatment of RP and retinal artery occlusion, and aim to have clinical trials in the near future (22,23). RP is an inherited progressive degenerative disorder characterized by the loss of rod photoreceptors, whereas retinal artery occlusion is an acute condition in which ischemia can lead to retinal and optic nerve damage. Since these conditions have very different etiologies, the evaluation of MANF to be neuroprotective in both could lend support for broader use. Ultimately, if MANF is beneficial for these devastating, but relatively rare ocular disorders, the next logical step is to see if the therapeutic value can be extended to help the leading causes of vision loss, macular degeneration and glaucoma.

Since MANF is neuroprotective, but not restorative on its own, it is likely that its greatest benefit will be its use as an adjunct in combination with another replacement or regenerative therapy. Indeed there are currently clinical trials underway for RP in a collaboration between The University of California-Irvine and jCyte using intravitreal injection of human retinal progenitors (24). If this cell replacement therapy is shown to be safe and effective, the addition of MANF may further enhance clinical outcomes. Finally, if the immune cell modulatory effects of MANF can be translated to other organ systems, it has the potential to be a therapeutically valuable factor in regeneration as a whole.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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